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THE ANNALS OF APPLIED BIOLOGY

THE OFFICIAL ORGAN OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

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Volume III 1916–17

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, MANAGER
LONDON: FETTER LANE E.C. 4
EDINBURGH: 100, PRINCES STREET
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WILLIAM WESLEY & SON, 28, ESSEX STREET, LONDON, W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
BOMBAY, CALCUTTA AND MADRAS: MACMILLAN & CO., LTD
TORONTO: J. M. DENT & SONS, LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

MARINE BIOLOGICAL LABORATORY WOODS HOLE LIBRARY MASS.

N497(4)

Cambridge:
PRINTED BY J. B. PEACE, M.A.,
AT THE UNIVERSITY PRESS

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THE FIG "CANKER," CAUSED BY *PHOMA CINERESCENS* SACC.

BY E. S. SALMON AND H. WORMALD.

Mycological Department, South-Eastern Agricultural College, Wye, Kent.

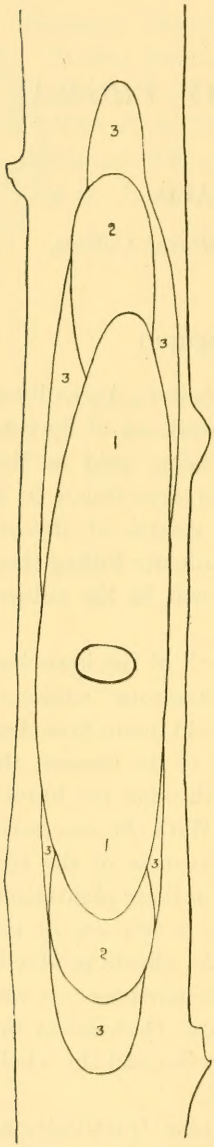
(With Plates I and II, and 1 Text-figure.)

IN 1914 complaints were received by the South-Eastern Agricultural College at Wye of serious diseases affecting the plantations of fig-trees in the district of Sompting, Sussex. On a visit being paid in May 1914 to the affected plantations, fig-trees of all ages were found to be suffering from fungous attacks of two kinds. A species of *Botrytis* was found on the tips of the branches, apparently gradually killing them back. This disease is still under observation and will be the subject of a further communication.

The second disease which occurred was a "canker" of the branches. In some cases, where old trees were badly attacked, numerous "cankers" were found, both on the younger branches, and on the old, main branches, often quite close to the ground. With the progress of the disease, the "canker" area enlarges, until the tissues extending through the branch are killed, with the result that the parts above die. With the successive removal of these "cankered" branches the productiveness of the tree is soon seriously impaired. The majority of the trees in these plantations are of the variety known as "Brown Turkey," but a few are of the "White Marseilles" variety. It was obvious from the effects produced that this "canker" disease was of serious economic importance; it was the opinion of the farmer of the largest fig-plantations that unless the cause of the disease could be discovered and a remedy devised the whole future of Fig-growing in that district was threatened.

The constant occurrence of a fungus with pycnidial fructifications on the cankered area was noted in the field, and the following notes were made from the examination of the material collected.

One of the "cankers" which may be taken as typical of those found on the larger branches is shown in the photograph at Fig. 1, Plate I, where the characteristic cracking of the bark in the older portions is seen. It will be observed that near the centre of the "canker" is the old base of a smaller branch and it is probable that that was the place where the fungus made an entrance. This particular canker was evidently the result of three distinct periods of activity of the causal organism. The oldest portion, recognized by the fact that the bark is very much cracked and separating from the wood, was elliptical and measured 16·5 × 5 cm. At each end of this ellipse the canker extended 5 cm. upward and 2·5 cm. downward, these extensions corresponding to the second period of activity. The check to the normal growth in thickness of the branch induced by the presence of the organism caused these affected areas to be depressed below the general surface of the branch. The youngest portions of the canker extended still further upward and downward and though they were but indistinctly marked off from the adjoining healthy portions of the branch they bore numerous pycnidia. The accompanying diagram of the canker shows the areas, affected during the three periods of activity, numbered respectively 1, 2 and 3; this diagram should be compared with Fig. 1, Plate I, which is a photograph of the same canker.



The portion of the branch bearing the "canker" was placed in a damp chamber and in twenty-four hours "tendrils" of conidia were issuing from the youngest portions of the canker but not from the rest. No conidia were obtainable from the older portions and there the fungus was either dead or had ceased to produce conidia, although these areas were still dotted over with old pycnidial pustules. The tendrils were at first of a pale orange colour but when fully protruded were almost or quite white. When a tendril or a portion of one is placed in a drop

of water the mucilaginous matrix binding the conidia together is dissolved and the conidia themselves stream apart and become diffused through the water; a slight "Brownian movement" is to be detected when the conidia are mounted in water.

Transverse sections through the bark show the fungal fructifications to be pycnidia. These bodies are more or less circular in outline and are from 250 μ to 600 μ in diameter; they are somewhat flattened and are therefore lenticular, appearing elliptical in transverse section. The pycnidia are produced a little below the surface, but on approaching maturity each develops a short neck which ruptures the outer layers of the bark and the tendril emerges through an apical pore. The wall of the pycnidium is lined with the conidiophores which are from 15 μ to 25 μ in length and which abstrict the conidia from their apices.

The conidia are continuous, ellipsoidal to fusiform, often with one extremity more rounded and broader than the other. Their dimensions are 6·5–13 × 2·4–3·6 μ. Usually they are about 9 × 3 μ and as a rule an increase of length is associated with a decrease in the width; thus the following dimensions are typical: 6·5 × 3·6, 9·2 × 3, 11·5 × 2·5, 13 × 2·1. With medium magnification they appear to be biguttulate, but by employing an oil-immersion objective this appearance is seen to be due to *two groups* of minute guttules. The two groups may merge into one another but usually they are quite distinct and situated at opposite ends of the conidium (see Fig. 10, Plate II).

The conidia are capable of germinating in water. When placed in a hanging-drop of distilled water and examined after remaining three days at the temperature of the laboratory (about 18° C.) a few of the conidia had germinated. They showed no appreciable increase in size before the protrusion of the germ tubes. The latter were at this stage one to four times the length of a conidium and frequently were more or less geniculate; they emerged laterally, sometimes at or near the middle of the conidium, at others more towards one or the other extremity, but none was seen truly polar.

In June of the same year (1914) Mr F. J. Chittenden sent us a specimen of the Fig "canker" from the glass-houses at Wisley; this bore pycnidia and conidia similar to those described above.

The pycnidial form of fructification and the spore-characters referred the fungus to the genus *Phoma*, and reference to Saccardo's *Sylloge Fungorum* enabled us to identify it with *P. cinerescens* Sacc. in *Mich.* I, p. 521 (1879). The specific diagnosis given is as follows: "Peritheciis gregariis, globoso-depressis, atrolivaceis, subcutaneis; sporulis

fusoideis, biguttulatis, 6–8 × 2–2·5, hyalinis. *Hab.* in ramis corticatis v. demum decorticatis *Fici Caricae*, in Italia et Gallia. Spermogonium *Diaporthes cinerescentis* Sacc."

To make certain of its specific identification, an example was sent to Prof. P. A. Saccardo, who replied, "J'ai examiné votre specimen. C'est sans doute mon *Phomopsis cinerescens*. Ma diagnose est incomplète, car les *sporophora* (comme il se fait très-souvent) étaient déjà disparus; aussi les *guttulae* dans votre specimen sont peu distinctes, mais cela est aussi variable." The author who transferred the present fungus from *Phoma* to *Phomopsis* was J. B. Traverso (in his *Flora Italica cryptogama*, vol. II, fasc. I, p. 278 (1906)), who after giving a description of *Diaporthe cinerescens* Sacc. merely remarks: "Status pycnidicus verisimiliter *Phomopsis cinerescens* (Sacc.) Trav. sporulis fusoideis, hyalinis, biguttulatis, 8 × 2." In general appearance and structure the present fungus agrees so well with other parasitic species which are still named *Phoma* that we retain it in that genus.

In searching for records of the occurrence of a Fig-tree "canker" in England, we met with the description given by Mr Massee first in the *Gardeners' Magazine*, for July 23 (1898), and later in his *Text-Book of Plant Diseases*, p. 431 (1903). In the former place Mr Massee wrote: "A disease presumably of old standing has of late years proved very injurious to fig-trees, and one remarkable feature in connection with this disease is the fact that it is most prevalent and destructive in those cases where the trees have received the greatest amount of attention, pruning more especially favouring its extension. The most usual symptoms of its presence are a cankered or ulcerated appearance of the bark, which frequently becomes eaten away in large patches, or variously cracked. In the majority of cases it is very evident that the canker first starts at a point where a branch has been cut away or accidentally broken off, and in all instances it appears that a broken surface of the bark is absolutely necessary to enable the fungus causing the disease to gain a foothold." While the general description of the disease given here agrees exactly with what we have found, the description of the fungus which Mr Massee gives as the causal organism is quite different from that of *Phoma cinerescens*. Mr Massee places his fungus in the *Melanconiaceae* ("perithecia absent; conidia produced on a more or less developed cushion or stroma formed beneath the surface of the matrix, and becoming erumpent") as a new species of the genus *Libertella*, viz. *L. ulcerata* Mass. In the *Gardeners' Magazine*, *l.c.*, it is stated: "Finally the fruit of the fungus, which is formed in

minute pustules, below the epidermis, bursts through to the surface, the exceedingly minute spores, only about 1 3,000 of an inch in length, oozing out through minute cracks in the epidermis, under the form of very slender, white, gelatinous threads or tendrils." The figure given of these spores is reproduced at Fig. 11, Plate II. It will be seen from Fig. 11, traced from Mr Massee's figure of the Fig canker *Libertella* in the *Gardeners' Magazine*, that the length of the conidia is about 1–3,000 of an inch (*i.e.* 8–9 μ), or approximately that of the conidia of *Phoma cinerescens* (compare Figs. 10 and 11, allowing for the difference in magnification). In *A Text-Book of Plant Diseases*[1] Mr Massee in describing his species under the name *Libertella ulcerata* Massee (sp. nov.) says, "Conidia fusiform, ends acute, continuous, curved, hyaline, 55 60 $\times$ 4 μ," and this description is repeated in his *Diseases of Cultivated Plants and Trees*[2]. This discrepancy in the accounts of the dimension of the conidia of *Libertella ulcerata* we are unable to account for. Our fungus, *i.e. Phoma cinerescens*, belongs to the *Sphaeropsidiaceae* ("perithecia containing conidia borne at the tips of slender conidiophores") and its conidia are ellipsoidal to fusiform, straight or slightly curved, ends usually rounded, averaging 9 $\times$ 3 μ, and never exceeding 13 μ in length.

The Herbarium at Kew was found not to contain the type specimen of *L. ulcerata* Mass. In March 1915, Mr Massee kindly forwarded to us an example of his fungus, writing "Enclosed is a fragment of the *type* specimen," and adding as a postscript, "I am almost certain that the *Libertella* is followed by a *Phoma* stage, but have not been able to get one from the other in cultures." The portion of the type specimen sent consisted of some hundreds of fructifications, mostly with dried-up tendrils of conidia still attached, and proved to be entirely *Phoma cinerescens*, with all the characters as described above. A thorough search of this type material showed no conidia resembling those described by Mr Massee, or any fructification of the type found in *Libertella*.

With reference to the parasitic nature of his fungus, Mr Massee states: "Experiments conducted on healthy young fig-trees show that the spores of the fungus—a species of *Libertella*—will not cause the disease when placed on the unbroken surface of even very young branches, whereas when the spores are placed on the end of a cut branch or on injured bark, inoculation always followed, and the mycelium was found in abundance at the expiration of 10 days. In one experiment

[1] Massee, G., *A Text-Book of Plant Diseases*, p. 431 (1903).

[2] *Idem*, *Diseases of Cultivated Plants and Trees*, p. 448 (1910).

a badly diseased branch, showing numerous threads of spores, was cut through; immediately afterwards the same knife was used for making an incision in the bark of a branch of a healthy young plant of *Ficus religiosa*. At the expiration of 10 days the wound showed undoubted symptoms of disease and at the end of five weeks the white threads of spores were found. This experiment proves that the disease may be imparted to healthy plants by using a knife that had previously been used for pruning diseased plants, and an examination of various diseased plants suggests the idea that this method of spreading the disease is not an unusual one."

Since the above work was not conducted with a pure culture of the fungus, and especially since we had found associated with the Fig "canker" disease a quite distinct fungus from that described by Mr Massee, it was important to obtain scientific evidence of the parasitism of *Phoma cinerescens*.

It was found that pure cultures could be obtained by two methods:

(1) A portion of a tendril obtained from the canker shown in the illustration (Plate I, fig. 1) was placed in a drop of sterilized distilled water; after dilution a small drop containing conidia was transferred to a tube of melted agar (prepared with an extract of prunes as nutrient) and a "poured plate" made. One such plate produced six sporelings; growth was rather slow and after eleven days the mycelial masses only measured from 4 to 7 mm. in diameter. From each of two of these a tube culture on an agar slant was prepared. In the tube cultures growth continued with more vigour and in seven days each showed a rather dense, flat, hyaline disc of mycelium about 2 cm. in diameter with a raised white ring of aerial hyphae midway between the point of origin and the periphery. Later these slant cultures consisted of a rich growth of dark brown mycelium with a few whitish tufts.

(2) An alternative method of obtaining cultures of the fungus was also adopted. The canker referred to above was cut across and transverse sections made through the discoloured wood. In this wood the vessels were found to be blocked with hyphae, tyloses and "wound-gum." Two small fragments of such sections made with a sterile razor were each transferred by means of a flamed platinum wire to an agar slant. The resulting growth resembled that obtained under similar conditions with mycelium produced from the poured plate of conidia, the white ring of aerial mycelium at a short distance from the point of inoculation again being a characteristic feature. The inference is that the mycelium in the affected tissues of the xylem is that of the

fungus seen on the exterior and indicates that it is possible to obtain a pure culture directly by transferring fragments of infected wood to a culture medium.

The medium used in these primary cultures was a decoction of prunes containing 1½ per cent. agar and although it was found to be quite suitable for vegetative growth, no reproductive bodies were produced under those conditions. Sub-cultures made on sterilized cylinders of potato in Roux's tubes and on slabs of wood (obtained by making transverse slices[1] of a branch from a fig-tree) placed in Petri dishes and autoclaved at 115° C. for 30 minutes, readily produced pycnidia and "tendrils" of conidia. On potato the pycnidia were numerous and in some cases well-developed "tendrils" emerged, while in others the conidia accumulated at the mouth of the pycnidium in a globular mass, the latter condition obtaining when the moisture within the tube was too great for the typical tendrils to retain their characteristic form. The "tendrils" and globules of conidia were at first of an orange colour changing later to a dark red. The conidia were similar to those obtained originally directly from the canker in their size, shape and guttulation.

On the slices of fig wood the fungus grew vigorously as a white mycelium and eventually produced a few scattered "tendrils": the latter were larger (stouter and usually longer) than those normally produced under natural conditions and were dark red in colour, the conidia however were similar to those obtained from the canker itself. It is interesting to note in this connexion that the "tendrils" were more numerous on the under surface of the wood, *i.e.* where it came in contact with the inner surface of the Petri dish, than on the upper free surface.

Inoculation Experiments on Fig-trees.

Young fig-trees in pots were obtained for inoculation with pure cultures of the *Phoma* and were kept in a greenhouse throughout the experiments.

Experiment I. In this preliminary experiment a plate culture of the fungus was started on June 8, 1914 as a sub-culture from one of the tube-cultures mentioned above as obtained from a "poured plate" of conidia. At the end of eight days (June 16) there was sufficient mycelium for use in the following inoculations:

[1] These slices were 4–5 cm. diam. and about 1 cm. thick.

(1) A V-shaped cut was made through the bark of a branch of one of the young fig-trees; the triangular tongue of bark was turned back and a little agar bearing mycelium was removed from the plate culture and inserted between the bark and the wood. The free portion of bark was then pressed back into its place and the wound was covered up with cotton wool and tinfoil to prevent possible infection from air-borne spores. This precaution was perhaps unnecessary for as shown in Exp. II exposed wounds remained uninfected.

(2) From another branch a small portion of the bark (about 1 cm. × 0·5 cm.) was entirely cut away and agar with mycelium placed on the exposed tissues, after which the wound was bound up as in the previous case.

On September 16 the coverings were removed and the following results observed:

(1) Round the inoculated wound was a depressed very dark elliptical portion of bark 2·5 × 1·2 cm.; the tissue bordering the canker was pale in colour and thus showed strong contrast with the sunken canker. The latter bore a few pycnidia which appeared on the surface as small protuberances with incipient "tendrils" consisting of conidia which on microscopic examination were indistinguishable from those obtained from natural cankers.

(2) The result of the second inoculation was similar to the one just described except that the canker was smaller, its dimensions being 1·8 × 0·8 cm.; tendrils with typical conidia were again produced.

In both cases the infected branch was seen in September 1914 to be slightly swollen when viewed in a direction perpendicular to the surface of the canker. No further increase in the size of these cankers was subsequently observed and in the following season both branches bore fruit and leaves. In February of the present year (1916) leaves and fruit again appeared but by the middle of March the secondary branch immediately above the canker of the second inoculation, and on the same side of the main branch as the canker itself, showed signs of wilting for the leaves began to droop and turn yellow, indicating that the transpiration current was interrupted in the neighbourhood of the canker.

Experiment II. Semicylinders of potato were placed in Roux's tubes and sterilized in an autoclave by steaming at a temperature of 115° C. for 30 minutes; they were inoculated from an agar slant culture on June 22, 1914. The fungus grew vigorously on the potato; pycnidia were readily produced and by July 15 "tendrils" of conidia began to

appear. Later the latter were further developed and on September 29 were used for a second series of inoculations on fig-trees. Two of the "tendrils" were removed on a sterile hooked platinum wire and placed in a small tube of sterile distilled water which was then agitated in order to diffuse the conidia throughout the liquid. Wounds are made on branches as in Exp. I, but instead of inserting agar and mycelium, a drop of the water containing the conidia was transferred to the wound by means of a pipette (previously sterilized) and allowed to flow in between the bark and the wood. In the case of control wounds a drop of sterile water only was inserted in a similar manner. Some of the wounds were bound round with damp cotton wool (previously sterilized in autoclave) and tinfoil secured by raffia in order to ensure favourable conditions for the germination of the conidia: the other wounds were left uncovered. In other cases again water containing conidia and fragments of tendrils was dropped on damp cotton wool which was then bound round certain internodes with tinfoil and raffia as before, but without injuring the bark.

Eight of the largest branches on two young fig-trees, var. White Marseilles, were selected and treated as follows, the branches being from 0·8 cm. to 2 cm. in thickness.

No. 1. Inoculated; wound covered with cotton wool and tinfoil.
„ 2. As in 1.
„ 3. Inoculated; wound left uncovered.
„ 4. Control; not inoculated but branch cut and wound covered with cotton wool and tinfoil.
„ 5. Control as in 4.
„ 6. Control; wound left uncovered.
„ 7. Spores placed on damp cotton wool which was then wrapped round an internode.
„ 8. As in 7.

Eight branches were similarly selected on two young trees of the Brown Turkey variety and respectively treated as those of the White Marseilles. Thus in this experiment six branches were wounded and inoculated, with an equal number of control branches also wounded, and in four cases conidia were applied to uninjured branches.

To test the viability of the conidia used in the experiment a drop of the conidia-containing water (as used in the inoculations) was used in preparing a "poured plate" which was then placed in the greenhouse in close proximity to the trees. No growth was to be observed in the

plate for several days but on October 9 (*i.e.* when ten days old) numerous small mycelial masses were conspicuous.

Positive results were obtained in each case where the conidia had been inserted in the wound. The rest all yielded negative results.

The trees were examined from time to time and the progress of the disease in the inoculated branches noted as follows.

White Marseilles.

No. 1. (Branch about 1 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. No visible canker: no discoloration of the bark but "tendrils" were seen in the neighbourhood of the wound: they were arranged in the form of an irregular ellipse 2·2 cm. × 1·4 cm., elongated in a direction parallel with the axis of the branch.

Jan. 25, 1916. Distinct sunken canker 4 cm. long and extending laterally three-quarters round the branch.

No. 2. (Branch about 2 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. „ „ „

March 25. A single tendril of typical conidia was seen at 2 mm. above the wound; otherwise there was no change externally.

April 20. The bark round the wound was slightly depressed below the general surface; the affected area measured 1·5 × 1·5 cm.

Jan. 25, 1916. Distinct sunken canker 1·5 cm. long and extending one-third round the branch.

No. 3. (Branch 1 cm. diam.)

Jan. 15, 1915. No distinct canker (*i.e.* no sinking or cracking of the bark) but round the wound and extending 1 cm. above and below and 0·5 cm. laterally were numerous small protuberances; one of these pustules removed and mounted in water proved to be a pycnidium containing conidia as described above.

Feb. 16. Tendrils were issuing from the pustules which now covered an area 3·5 × 1·8 cm.

Jan. 25, 1916. Distinct sunken canker 5 cm. long and extending three-quarters round the branch.

Brown Turkey.

No. 1. (Branch 1 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. „ „ „

Mar. 3. A rather indistinct zone of discoloured bark (darker than the rest) was observed at 3–5 cm. from the wound.

Mar. 25. A single tendril of typical conidia was found at 2 mm. from the point of inoculation.

Jan. 25, 1916. Distinct sunken canker 2 cm. long and one-third round the branch.

No. 2. (Branch 0·8 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. The bark was discoloured for 2–3 mm. round the wound; at 4 mm. from the point of inoculation was a small group of pustules.

Mar. 3. A tendril was observed projecting from one of the pustules; it was removed and microscopic examination showed conidia as before.

Jan. 25, 1916. The affected area, now 2 cm. long and extending three-quarters round the branch, was only slightly depressed below the general surface but was distinguished from the rest by its darker colour.

Feb. 24. Branch dead above the canker, as shown by absence of any growth, the rest bearing leaves and young fruit.

No. 3. (Branch 1 cm. diam.)

Jan. 15, 1915. No external change.

Feb. 16. No canker and no discoloration noticeable but round the wound was an irregular circle (about 1 cm. diam.) of tendrils.

Jan. 25, 1916. The affected area appeared as a slightly sunken canker 2 cm. long and extending half-way round the branch.

Thus in the two series of experiments eight inoculations through wounds were made in all, and in every case not only was there a "canker" formed round the point of inoculation but the fruiting fungus appeared in the affected tissues and produced tendrils of conidia resembling those found on "cankers" arising from natural infections. Since "cankers" were so readily induced when the fungus, either in the form of conidia or of mycelium, was introduced into the internal tissues through wounds, the negative results in those cases in which conidia were applied to the uninjured bark indicate that in all probability the fungus is solely a wound-parasite.

Preventive Measures.

From our knowledge of the biology of the parasite, it is very unlikely that spraying with any fungicides will hold the disease in check. The direct measures should consist of a search for all "cankers," and the cutting of them out, down to the sound tissues, at a time when the disease is dormant; it will be advisable to paint over the wounds made with Stockholm tar. All dead branches must be cut out and *promptly burned*, as otherwise the fungus producing conidia on the dead wood might constitute a dangerous source of infection. The indirect measures

which should be employed—and these will be no less important than the direct measures—must consist of the prevention as far as possible of wounds to the bark. The making of wounds by the boots of men climbing in the branches when gathering the figs must be rigorously prevented, as well as those caused by careless hoeing or digging round the trees or due to the attacks of animals. The minimum amount of pruning to the older branches should be employed until the disease has been stamped out or reduced to a small amount.

DESCRIPTION OF PLATES I, II

PLATE I

1. Canker on a large branch of a fig-tree. $\frac{2}{5}$ natural size.

2. Lower half of the same canker, natural size, showing the small wart-like protuberances produced by the pycnidia.

3. Section through the bark of a canker; the pycnidial form of the fructifications is evident. ×60.

PLATE II

4. Portion of a canker with "tendrils." ×4.

5. Transverse section, natural size, through the middle of the canker shown in Fig. 1; the affected tissues were dark brown in colour.

6. *Phoma cinerescens* in pure culture on sterilized potato, natural size.

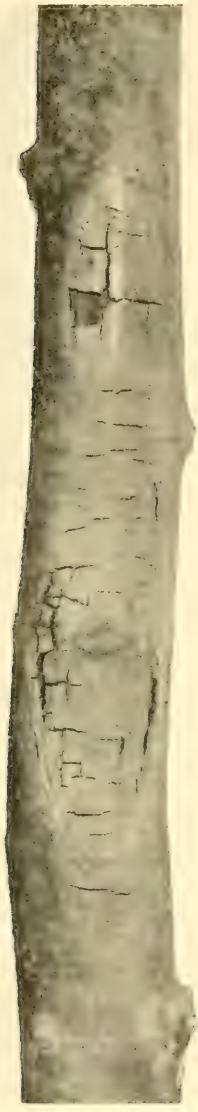
7. Two "tendrils" of *Phoma cinerescens* produced in pure culture on a slice of sterilized fig wood. ×4.

8. Branch of fig-tree after inoculation with conidia taken from a pure culture, natural size; an irregular ring of "tendrils" at some distance from the wound is shown.

9. Another branch after inoculation with conidia; the protuberances produced by the pycnidia are seen around the wound.

10. A camera-lucida drawing of conidia of *Phoma cinerescens*; each conidium is provided with two groups of guttules. ×1000.

11. Drawing traced from Mr Massee's illustrations, in the *Gardeners' Magazine*, of the conidia of the Fig canker *Libertella*. ×500.



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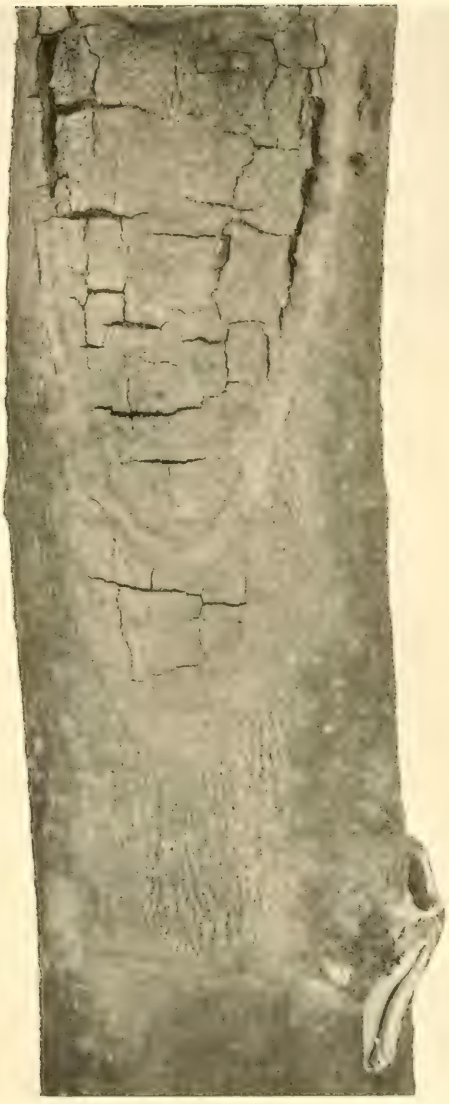

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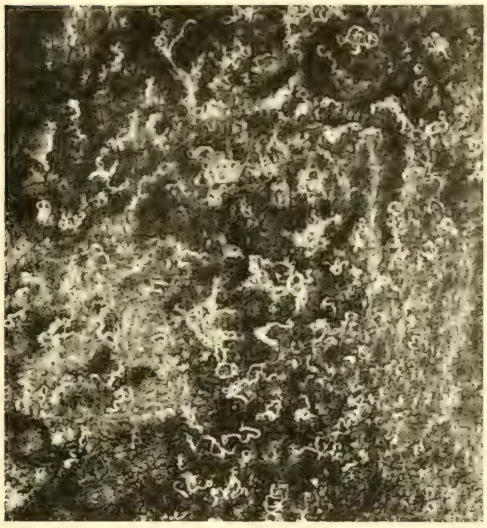

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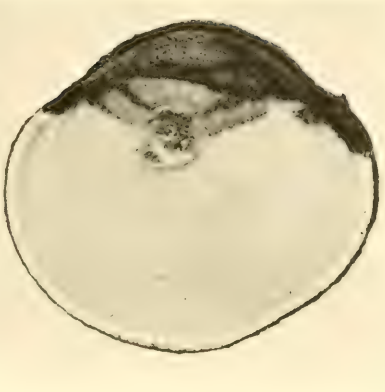

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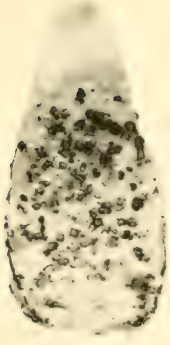

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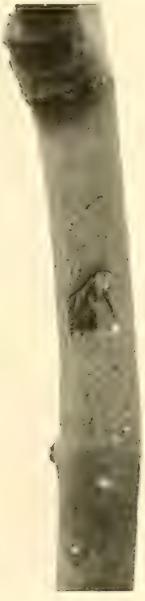

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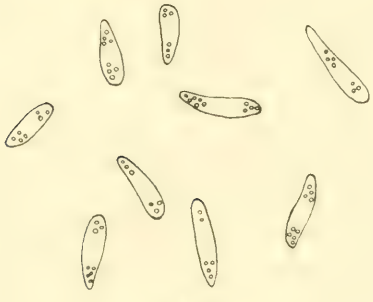

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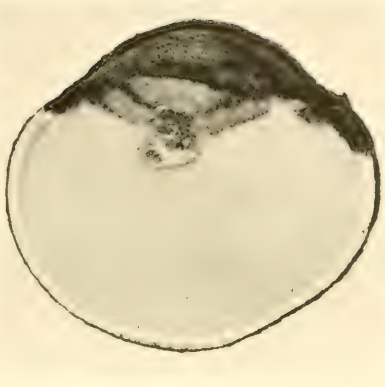

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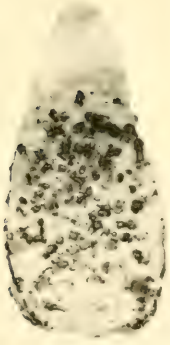

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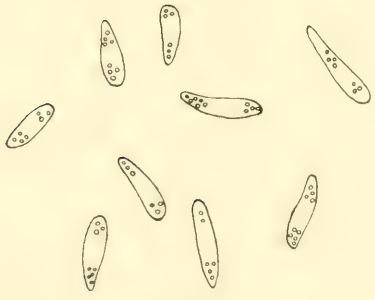

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11

SHRINKAGE, SWELLING AND WARPING OF CROSS-GRAINED WOODS: No. 1, YANG (*DIPTEROCARPUS* SP.).

By PERCY GROOM,

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(With a Diagram.)

No detailed investigations appear to have been conducted on the changes of dimensions and shape occurring during the wetting and drying of cross-grained woods. Yet such research bears upon problems that are of scientific as well as industrial importance; for many timbers growing in hot countries are cross-grained, that is to say, their fibres and other constituents in successive concentric layers round the longitudinal axis are directed alternately in right-handed and left-handed spirals. Moreover, a number of such timbers are of commercial importance.

The statistics available show that when straight-grained woods dry from their fresh (green) condition to an air-dried (ordinary seasoned) state the percentage shrinkages of their rods are as follows when cut: longitudinally, usually ·1 (·01–·8); radially, usually 3–5 (1·1–6); tangentially, usually 6–10 (1·8–10·5).

At first thought it might be anticipated that a cross-grained timber would show increased longitudinal but decreased transverse shrinkage or swelling, because of the existence of transverse and longitudinal components respectively. The results obtained do not bear out such an anticipation.

In this investigation the Indo-Malayan cross-grained commercial timber known as Yang, *Dipterocarpus* sp. (possibly *D. tuberculatus*), was used. When first imported into England it was tested as a substitute for teak (in window-cills, doors, etc.), and was sometimes

sold as "yang teak," but the results were not satisfactory. In-doors the wood twisted as it dried; while out-of-doors when it absorbed moisture yang was described as speedily showing on its surface a "white growth" and as undergoing early decay. During the progress of this investigation the practical problems of preventing these defects were more or less solved, and a digression on the cause of the disfigurement and decay out-of-doors may not be out of place.

Submersion of yang boards under water causes quantities of white substance to appear at intervals over the surface and especially at the ends. This substance is mainly resin that is forced out by the swelling of the woody tissue. I examined a few minute quantities of the substance dissolved in methylated spirit, and in each there occurred a microscopic fungal mycelium. When the sodden yang wood was allowed to dry the white substance remained as a disfiguring chalk-like exudation. When, in turn, a very little methylated spirit was rubbed over the surface of such dry wood, the disfigurement vanished as the resin dissolved and covered the surface with a film of varnish (recalling French polish in its effect). Practical trial alone can show the extent to which this treatment will preserve this wood from decay, or be of service in connexion with other resinous dipterocarp woods.

In the investigations the pieces of yang wood used were in the form of radially and tangentially cut boards, about 48–61 cm. in length, 12–13 cm. in width, and 1·35 cm. in thickness. In order to induce and measure the swelling they were submerged under water and measured at intervals. Thereafter they were allowed to dry at ordinary temperatures in the laboratory, and measured at intervals. The first measurements were made by means of a wooden rod graduated only to millimetres, but subsequently use was made exclusively of vernier callipers accurate to ·02 mm. The changes of curvature were recorded graphically by an instrument which Professor Dalby was so kind as to design for the purpose. The instrument is described in a separate paper, in the present issue.

General Results.

The most interesting results concerned the changes in length of the wood during the absorption and emission of water. In both cases a shortening and a lengthening took place. During the absorption of water the radial and tangential boards showed successively:

First, an increase of length together with relatively small increase of width. This latter, in the one case tested, was most marked near

the ends of the board, because the water was entering mainly through these.

Second, the boards actually shortened while at the same time they continued to widen.

Third, once more lengthening took place and was accompanied by widening.

During drying the reverse phenomena wholly or in part were exhibited; for the shortening at the beginning was succeeded by elongation, which was final in one case (yang 2) but was followed by lengthening in the case of yang 1; in all these cases the board continued throughout to shrink in width (excepting for occasional slight deviations).

Yet throughout these contrary changes in length the board continued to increase in surface during the absorption of water, and to decrease in surface during drying. And, in connexion with the explanation of the phenomenon, it is worthy of note that the board (tangential, yang 2) which showed the smallest ultimate decrease in length and most persistent elongation during drying yet experienced the greatest decrease in surface.

The most probable explanation of the reversal in change of length during the absorption of water appears to be one akin to that offered by Professor Brereton Baker, who suggested the analogy of a net-work or lattice-work in which swelling takes place more rapidly along the length than at right angles to this. In this case the meshes formed by the crossing vessels, fibres, and so forth are lozenge-shaped, narrow, and elongated along the axis of the tree-trunk (and board).

If we imagine two fibres or tracheae *ABC* and *DBE* intersecting at *B*, and consider the two sides *BC* and *BE* of the triangle *CBE*, then as water is absorbed by the board it is taken in most rapidly at the ends and travels more rapidly (in the vessels) along *ABC* and *DBE* than in any other direction. The result is relatively considerable elongation of *BC* and *BE*, with relatively inconsiderable increase in the angle *CBE*. Increased distribution of the water at right angles to *BC* and *BE* next causes more rapid expansion in those directions, tending to cause the two sides to rotate outwards about the centre *B* and thus cause a widening of the base of the triangle but a shortening of the perpendicular from *B* to that base. This would also take place if the sides elongated to a disproportionately small extent. Thereafter elongation of the two sides associated with disproportionately less expansion at right angles would cause a reversal of the immediately

preceding phase. Through these three phases the board named yang 2 passed, but during the final stage of elongation there were fluctuations, which possibly were due to the successive transverse belts or zones of the board at different distances from the ends being at different phases, and to irregularities in the rate of distribution of moisture in the board. Dotted lines in Diagram 1 illustrate, on a very exaggerated scale, the paths traversed by the points *C* and *E* during the absorption of water.

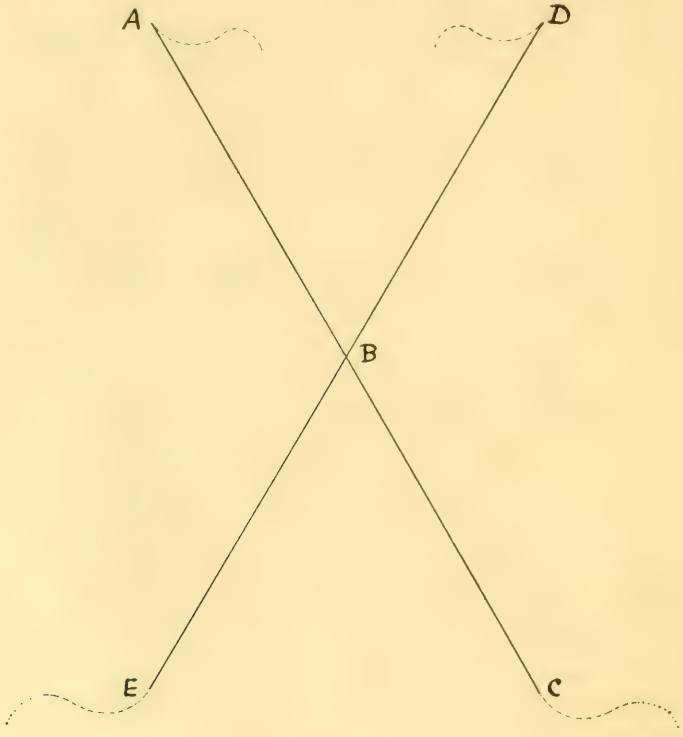

Diagram 1

When the board is drying and shrinking the reverse changes tend to take place, as drying is most rapid at the ends and presumably so along the lines of vessels. Yang 1 (tangential) went through such changes completely, but yang 2 (tangential) and the radial board showed merely the initial decrease and the succeeding increase in length; both associated with decrease in width. Dotted lines in Diagram 1 represent on a very exaggerated scale the paths of the points *D* and *A* during drying.

There is no absolute certainty that during soaking the boards had attained their maximum dimensions, though it is probable that such was at least approximately the case. The tangential board, yang 1, had certainly attained its greatest length; and judging by the lengths of the diagonals and widths so likewise had yang 2. When, as a result of drying, the boards had shrunk to their minimal recorded dimensions, these may be assumed to represent the true minimum sizes in relation to the surrounding atmosphere (dry air of the laboratory).

The changes in length were small or exceedingly small ultimately, being only ·012–·075 % of the maximum length. The changes in width at the ends were: tangentially, 5·92–6·29 % during drying, and 6·98 % (in the one case measured) during soaking; radially, during drying 5 % of the maximum width.

The subjoined Table gives the percentage changes of the (maximum) linear dimensions.

	Tangential Boards			Radial Board	
	Yang 1		Yang 2		
	Water absorbed 313 grammes	Water exhaled 307 grammes	Water exhaled 209 grammes	Water absorbed 316 grammes	Water exhaled 331 grammes
In length:					
maximum	—	·075	·030	—	·037
ultimate	·079	·063	·012	—	·020
In width:					
at ends	6·980	5·920	6·29	(5·5)	5·0
middle	7·400	5·980	—	—	—

The shrinkage in both length and width was less in all cases than the previous swelling, and this was so even when (in the radial board) more water was lost than had originally been absorbed. This permanent expansion of pieces of wood after soaking in water appears not to have been recorded, and it remains to be seen whether or no straight-grained woods show the same behaviour. That some change takes place in wood by the prolonged action of water at high temperatures (steam) is well known, and reveals itself in permanent decrease of elasticity and strength.

Although during drying and soaking respectively lengthening and shortening may take place, the area of the faces of the board always decreases and increases respectively during those processes. So much does the change in width correct any reverse change in length that the board yang 2 when drying lost in area to a greater extent than did the other boards despite of the fact that its ultimate decrease in length was much the smallest.

One marked character of at least some cross-grained woods is their liability to twist during wetting and drying, and the tangential boards of yang displayed this character. The two tangential boards of yang showed on their faces the grain running obliquely apparently in the same sense in each board, but in opposite senses in the two boards. Thus on each face of yang 1 the grain tended to run from the right hand to the left hand down the board, whereas in yang 2 it tended to run from left to right downwards. Considering either of these boards on one face the one diagonal running rather with than across the grain changes relatively slightly in length as the board becomes wetter or drier, whereas the other diagonal on the same face changes more in length becoming concave when drying or convex when wetting: the diagonals superposed on these on the reverse face behave in an opposite manner. The result is that each face of the board becomes convex along one diagonal, and concave along the other; that is to say a twisting movement results from the establishment of a couple. That this explanation is correct is confirmed by the fact that the twist is in the opposite sense during the swelling and drying of the same board; and is in the opposite sense in the two boards yang 1 and yang 2 when these are undergoing the same kind of change of water contents, whether this change be drying or wetting.

Moreover if such be the true explanation of the twisting a radially cut board should not exhibit this behaviour. The radially cut yang board showed no trace of twisting either when wetted or dried. These facts suggest, on the one hand, that possibly the twisting of so-called straight-grained boards in general is due to a less marked and differential obliquity on the two faces, and on the other hand that cross-grained woods now excluded from commercial use by reason of their twisting propensities could be utilized if they were cut into radial ("quartered," "rift-sawn") boards. The facts also emphasize the advisability of seasoning cross-grained woods not only thoroughly, but also slowly, the latter because the extent of the twist is largely influenced by the rate of drying of the surface.

In a transverse direction the tangential boards at their ends remained nearly straight, the very slight amount of curvature possibly conforming with the moderate degree of tangential shrinkage or swelling.

Observations on Yang 1.

This board was tangentially cut, its length and width slightly exceeding 61 cm. and 12·85 cm. respectively.

The two faces were labelled *y* and *Y* respectively, *Y* and *Yx*, *y* and *yx* denoting the four corners of what may be termed the top of the board, *y* and *Y* being at the same edge or corner: similarly *Yb* and *Yxb*, *yb* and *yxb* denoted the four corners at the bottom end of the board. Consequently the lengths of the board on the two faces are represented by measurements *y . yb*, *yx . yxb*, *Y . Yb*, *Yx . Yxb*, of which *y . yb* and *Y . Yb* represent the same margin of the board on two different faces, as do *yx . yxb* and *Yx . Yxb*. The diagonals were thus *Y . Yxb*, *Yx . Yb*, *yx . yb*, and *y . yxb*.

In this cross-grained board on each face the grain runs from the top towards the bottom in a direction from right to left hand, though on face *Y* the grain is to a considerable extent nearly straight on the lower (bottom) half.

Observations. Set A (Table A).

The first measurements of yang 1 were made on a board, already partially seasoned in the timber-yard, during its drying in the laboratory. As a simple measuring rod was used the longitudinal changes in length were too small for accurate estimation, but the changes in length of the diagonals and in width were sufficiently considerable for approximate measurement and are recorded in the accompanying Table A.

In accordance with the direction of the grain the shrinkages along the two diagonals *Y . Yxb* and *yx . yb* were greater (·2 and ·15) than along the other two diagonals, so that the board became concave along the former pair and convex along the latter pair. The two ends of the board were thus twisted so as to lie in different planes, but they nevertheless retained their transverse straightness approximately.

Table A.

Date	Weight in grammes	Diagonals in centimetres				Width in centimetres	
		Y . Yxb	*Yx . Yb*	*y . yxb*	*yx . yb*	Top	Bottom
April 28	761·4	60·7	59·7	61·1	61·1	12·9	12·85
„ 30	751·2	—	59·65	61·05	61·05	12·8	12·7
May 3	—	60·6	59·6	61	61	12·7	12·6
„ 10	733·54	60·55	59·625	61·05	61	12·75	12·6
„ 19	738·85	—	—	—	—	12·75	12·6
June 1	725·35	60·5	59·6	61	60·95	12·7	12·525
Total difference	36·05	0·2	0·1	0·1	0·15	0·2	0·325

Set B (Tables B I and B II.)

The next set of measurements (*B*) were made on the board as it was compelled to swell by submersion under water: vernier callipers and Professor Dalby's instrument being used for the observations.

The main results as evidenced by the statistics given in Tables B I and B II, and as proved by the graphic records (unpublished) of the curves, are:

The absorption of water at first causes a sudden relatively considerable increase in length, which is relatively more marked than the increase of width even at the ends. This is succeeded by a decrease in length, which is synchronous with a more rapid gain in width.

The water enters most largely by the ends; the gain in width is marked here at first, and only after some time does the middle of the board display a similar full rate of widening.

As water continues to be absorbed the board, after undergoing its peculiar shortening, gradually lengthens and attains its maximum length even before it has absorbed the maximum amount of water, after which one or more times a shortening was noticeable. These contrary fluctuations as regards length are probably to be attributed to the fact that as the water penetrates from the ends the transverse belts at increasing distances from the two ends successively go through the shortening and lengthening phases, so that the measured result as regards the whole board is the algebraic sum of a number of items that are unequal and at times opposite in sense.

Widening of the board continued as long as water was absorbed, but the slightness of the increase in width towards the conclusion of the soaking strongly suggests that the maximum width had been very nearly reached.

Table B I records the changes in linear dimensions of yang 1 during the absorption of water. The results of these measurements are arranged in Table B II, by grouping together measurements made at short intervals on the same day, and by taking the averages of the longitudinal, transverse end, and transverse middle measurements.

In reference to these Tables it was conceivable that two sources of misinterpretation were possible. On the one hand curvature or straightening of the board might cause a spurious appearance of shortening or lengthening respectively. On the other hand the interval necessarily intervening between the weighing of the board and the recording of the measurements and curves would lead to a certain degree

TABLE B I. *Absorbing water.*

Date	Hour	Weight in grammes	Difference	Longitudinal *yx.yb*	Difference	Longitudinal *y.yb*	Difference	Tangential *y.yx*	Difference	Tangential *yb.yxb*	Difference	Tangential Middle	Difference	
VI. 21	12.30	721·650	—	61·026	—	61·032	—	12·710	—	12·540	—	12·478	—	After measurement left in the damp air of a hot-house until VI. 22. 10
„ 22	10	738·1	16·45	„ ·050*	·024	„ ·054*	·022	„ ·838	·128	„ ·662	·122	„ ·562	·084	After measurement placed in water for 2½ hours
„ „	2.15	797·25	59·15	„ ·072*	·022	„ ·076*	·022	13·452	·614	„ ·857	·195	„ ·592	·030	
„ 23	11.15	841·5	44·25	„ ·036	−·036	„ ·030	−·046	„ ·624	·172	13·320	·463	„ ·856	·264	
„ „	3.15	842·6	1·1	„ ·039	·003	„ ·034	·004	„ ·628	·004	„ ·345	·025	„ ·902	·046	Measurements begun at 3.15 and continued until 4.15, when weighing took place
„ 24	11.15	881·7	39·1	„ ·052	·013	„ ·058	·024	—	—	(„ ·414)	—	—	—	
„ „	12·10	874·3	−7·4	„ ·052	·0	„ ·069	·011	„ ·624	−·004	„ ·419	·074	13·160	·258	After 12.10 submerged, then (at approximately 2.35) measured, curves taken, and finally weighed at 3.30
„ „	3.30	873·6†	−·7†	„ ·055	·003	„ ·060	−·009	„ ·626	·002	„ ·424	·005	„ ·180	·020	
„ 25	11.15	901·6	28·0	„ ·060	·005	„ ·072	·012	„ ·622	−·004	„ ·432	·008	„ ·324	·144	
„ „	12.30	891·5	−10·1	„ ·058	−·002	„ ·074	·002	„ ·620	−·002	„ ·426	−·006	„ ·330	·006	
„ 28	10.15		46·6	„ ·073	·015	„ ·102	·028	„ ·630	·010	„ ·472	·046	„ ·434	·104	Measurements, curves etc. begun at 10.15, weighed at 11.45, then renewed measurements begun at 12
„ „	12	938·1		„ ·072	−·001	„ ·086	−·016	„ ·628	−·002	„ ·470	−·002	„ ·434	·0	
„ 29	11.20	967·3	29·2	„ ·074	·002	„ ·082	−·004	„ ·630	·002	„ ·474	·004	„ ·440	·006	Measured then weighed, then curves taken, then measured again
„ „	12.20			„ ·072	−·002	„ ·082	·0	„ ·630	·0	„ ·472	−·002	„ ·440	·0	
VII. 1	10.15			„ ·076	·004	„ ·090	·008	„ ·634	·004	„ ·482	·010	„ ·446	·006	Measurements and curve-taking begun at 10.15, weighed at 11.57, then measurements remade
„ „	12	986·6	19·3	„ ·073	−·003	„ ·088	−·002	„ ·632	−·002	„ ·480	−·002	„ ·445	−·001	
„ 5	11.55	1017·1	30·5	„ ·072	−·001	„ ·086	−·002	„ ·648	·016	„ ·494	·014	„ ·472	·027	
„ 7	11	1034·6	17·5	„ ·072	·0	„ ·085	−·001	„ ·645	−·003	„ ·502	·008	„ ·478	·006	
Total difference			312·95		·046		·053		·935		·962		1·000	
Percentage difference of the final dimensions			—		·075		·087		6·85		7·1		7·4	

* These numbers were originally recorded as 60·5, 60·522, 60·504, and 60·526 but I feel sure that I recorded in each case ·5 in place of ·05; hence the alterations of the original records.

† The comparative estimated numbers are 880·9 and +6·3.

TABLE B II.

Date	Hour	Weight in grams.	gain	Length in cm.	difference	Width of ends in cm.	difference	Width of middle in cm.	difference	Length × average width of the ends	Area = length × average width	Increase of area per 10 grammes of water absorbed, when estimated by length × average width of ends	Increase of area per 10 grammes of water absorbed, when estimated by length × average width of whole board
VI. 21	—	721·65	—	61·029	—	12·625	—	12·478	—	770·491	765·975	—	—
„ 22	10	738·1	16·45	„ ·052	·023	„ ·750	·125	„ ·562	·084	778·413	772·674	4·8	4·07
„ „	2.15	797·25	59·15	„ ·074	·022	13·154	·404	„ ·592	·030	803·367	786·206	4·2	2·3
„ 23	—	842·05	44·8	„ ·035	−·039	„ ·479	·325	„ ·879	·287	822·691	804·380	4·3	4·06
„ 24	12.10) 3.30)	874	31·95	„ ·059	·024	„ ·523	·044	13·170	·291	825·701	814·893	0·94	3·29
„ 25	—	896·55	22·55	„ ·066	·007	„ ·525	·002	„ ·327	·157	825·918	819·872	0·096	2·2
„ 28	—	938·1	41·55	„ ·079	·013	„ ·550	·025	„ ·434	·107	827·620	824·078	0·41	1·01
„ 29	—	967·3	29·2	„ ·077	−·002	„ ·551	·001	„ ·440	·006	827·654	824·234	0·012	0·05
VII. 1	—	986·6	19·3	„ ·082	·005	„ ·557	·006	„ ·445	·005	828·089	824·668	0·22	0·22
„ 5	—	1017·1	30·5	„ ·079	−·003	„ ·571	·014	„ ·472	·027	828·903	825·849	0·266	0·387
„ 7	—	1034·6	17·5	„ ·078	−·001	„ ·573	·002	„ ·478	·006	829·011	826·080	0·06	0·13
Total difference		—	313·	—	·049	—	·948	—	1·00	58·52			
Percentage of the final dimensions		—	—	—	·079	—	6·98	—	7·4	7			

The swelling per cent. in a tangential direction is 91 times that in a longitudinal direction.

of inaccuracy as to the exact amount of water present in the board at the time of the records. Both these objections are shown in the succeeding remarks to be groundless as regards the essential results obtained.

Rate of loss of water during measurement and recording of curves. The board was first left in very damp warm air for nearly 24 hours (VI. 21–22); on June 22nd during measurement and so forth from 10 a.m. to 11.45 a.m. it lost water at the rate of 1·7 grammes per hour. In this case the water was wholly hygroscopic and in the cell-walls. On other days in summer in the dry laboratory air the board lost water during measurement etc. in most cases at the rate of 7·2 grammes per hour (VI. 22 from 2.15 to 3.5 p.m., and from 3.5 to 3.30 p.m.; VI. 23 from 11.15 a.m. to 12.5 p.m.; VI. 25 from 11.15 a.m. to 12.30 p.m.), though on one day (VI. 24 from 11.15 a.m. to 12.15 p.m.) the rate was 8·1. In these cases it may be assumed that the loss of water was largely at least at the expense of that present in the lumina of the wood, and at the surface of the sides. These figures render possible an approximately accurate estimate of the amount of water present in the wood at the middle of the time occupied in recording measurements and curves on any day.

In regard to the estimates of the amount of water in the board and the length the following cases may be discussed. On June 24th observations were made in the following order: at 11.15 a.m. the board was weighed (881·7 g.), then curves and measurements were taken until 12.10 p.m., when the board was once more weighed (874·3) and a second set of curves and measurements were immediately taken. When these last were concluded the approximate weight would be $874{\cdot}3 - 7{\cdot}3 = 867$ g. The board was then submerged until 2.30 p.m., when curves and measurements were made before it was weighed (873·69) at 3.30, so that at 2.30 p.m. its approximate weight was 880·9. But whether these corrected or the recorded weights be accepted the changes in linear dimensions are not proportional to the change in the amount of water contained. During this phase these dimensions are determined rather by the exact distribution than by absolute amount of the moisture, probably because the water is largely passing from the lumina into the cell-walls whether the wood be soaking or drying.

Again on June 28th, 29th, and July 1st, to begin with the measurements and curves were taken, then the board was weighed, after which a second set of measurements and curves were made. This second set therefore represents the wood in a drier condition (11, 7·3, and 12·5 grammes of water respectively having been lost) so that the linear

dimensions are smaller than in the first set. Accordingly the average between the two sets of measurements represents more accurately the linear dimensions at the recorded weight.

The subjoined Table B III reports the changes of curvature lengthwise of yang 1 (during the absorption and exhalation of water) along the two sides *y . yb* and *yx . yxb*.

TABLE B III.

	y . yb					*yx . yxb*				
	Date		End *y*	Middle	End *yb*	Date	End *yx*	Middle	End *yxb*	
VI.	21	2	*vs*	*c* (2)	*c*	VI. 21	(*vs*)	*c* (3)	*c*	Absorbing water
,,	22	11.40	*S* to *cs*	*c* (3)	*c*	,, 22 11.30	*S* to *cs*	*c* (3)	*c*	
,,	,,	2.25	*c*	*c* (5)	*c*	,, ,, 2.30	(curve defectively taken)			
,,	23	11.15	*cs*	*c* (4)	*c*	,, 23 11.30	(circa) (*vs*)	—	*cs*	
,,	,,	4.10	*cs*	*c* (4·25)	*c*	,, ,, 4.15	*v*	*vs*	*vs* to (*cs*)	
,,	24	11.46	*Sn*	*Sn*	*Sn*	,, 24 11.30	*S*	*S*	*S*	
,,	,,	3.10	*Sn*	*Sn*	*Sn*	,, ,, 3.35	*S*	*S*	*S*	
,,	25	11.15	*Sn*	*Sn*	*Sn*	,, 25 11.45	*S*	*S*	*S*	
,,	28	11 to 12	(*vs*) to (*cs*)	*cs* (·75)	—	,, 28 11.45	*S*	*S*	*S*	
,,	29	11.55	*S*	*S*	*S*	,, 29 11.50	*S*	*S*	*S*	
VII.	1	11.10	*v* (·9)	*S*	*S*					
,,	5	11.40	*v* (·75)	*S*	*S*	VII. 5 11.35	*S*	*S*	*S*	
,,	7	11.17	*v* (2·25)	(*v* 2)	*v*	,, 7 11.25	*S*	*S*	*S*	
,,	9	12.53	(A wavy concave to straight line)			,, 9 12.45	*S* (*vs*)	*S*	*S*	Drying
,,	13	12.6	*c*	*c* (1·5)	*c*	,, 13 12.10	*c*	*c* (3·25)	*c*	
,,	15	11.57	*c*	*c* (1·5)	*c*	,, 15 11.50	*c*	*c* (3·25)	*c*	
,,	16	11.17	*c*	*c* (1·5)	*c*	,, 16 11.30	*c*	*c* (3·25)	*c*	

The first four columns refer to *y . yb*, the others to *yx . yxb*. Columns 1 and 5 give the dates and times of observation. The other columns denote the curvature: and as the curve of each side is often not uniform along its whole length, each column headed "middle" refers to the curvature at the middle of the length, while the remaining columns headed "end" give the curvatures at the end of each of the two halves of the length. In the columns: *c*, *cs*, and (*cs*) respectively mean "concave," slightly so, or very slightly so; *v*, *vs*, (*vs*) respectively mean "convex," slightly so, or very slightly so; *S* and *Sn* mean straight and nearly straight respectively; the number succeeding in brackets in columns gives the number of millimetres of the middle above or below the straight or base line.

The statistics given in Table B III when compared with those of Table B I show in particular that the unexpected shortenings of the

board during the absorption of water are true, and not due to increased curvature. This is true of the changes in length between June 22nd and 23rd, and July 1st to 9th.

Changes in transverse curvature. In like manner Table B IV shows on comparison with Table B I that the measurements recorded of the widths are not determined by the transverse curvatures; even when there is a considerable difference in the amount of widening of the two ends (June 25th to 28th) this is not associated with any corresponding difference of curvature.

Table B IV.

Date and times	*yb . yxb* Change of shape	*yb . yxb* Change of width	*yx . y* Change of shape	*yx . y* Change of width	
VI. 21	Concave		Concave		Absorbing Water
„ 22 10.30–10.45	Less concave (tends to straighten)	·122	Less concave (tends to straighten)	·128	
„ „ 3–2.50	More concave (curves)	·195	*Vigorously* convex in middle (curves equally)	·614	
„ 23 11.50–12	More concave throughout (curves)	·463	Less convex (tends to straighten)	·172	
„ 24 3.5–3	Concave throughout	·104	Still less convex (tends to straighten)	·002	
„ 25 12.30–12.20	Nearly straight (straightens)	·005	Minutely convex (tends to straighten)	−·005	
„ 28 10–10.5	„ „ as a whole minutely *concave*, nearly straight	·043	„ „ as a whole minutely *convex*, nearly straight	·009	
„ 29 11.35–11.40	„ „	·002	Straight	·0	
VII. 1 11.45–11.57	„ „	·007	Minutely convex	·003	
„ 5 11.45–11.50	„ „	·015	Nearly straight	·013	
„ 7 11.12–11.7	Practically straight	−·008	Practically straight	−·003	
„ 13 12.40–12.45	Slightly convex (curves)	·586	Became concave (curves more)	·631	Drying
„ 15 12.12–12.10	Minutely concave (curves)	·034	Slightly more concave (curves more)	·024	
„ 16 11.10–11.16	Definitely concave „	·058	Concave, about = VII. 13; hence it straightens slightly	·034	

Set C (Tables C I and C II).

The subjoined Table C I records the linear changes of dimensions of the same board (yang 1) during the process of drying from an approximately saturated condition to a state of aqueous equilibrium with the air of the laboratory. Table C II gives the averages of the results obtained.

In addition to details that will be discussed subsequently in comparing swelling and shrinkage, two facts are worthy of note.

1. During shrinkage there is reached a certain phase at which the board elongates while still decreasing in width. This phase, when judged by the amount of water contained, is not exactly synchronous with the corresponding reverse one during swelling. As in the latter case this reversal in the change of length is temporary and is succeeded by longitudinal shrinkage that brings the board down to its earlier shortened condition.

2. Whereas the board when absorbing water at first widens more rapidly at the ends than in the middle, when drying it contracts in width more rapidly at the middle than at the ends. Two possible explanations of this latter fact present themselves. (*a*) When drying owing to the more rapid conduction along than across the grain there is near the ends a rapid shortening which tends to cause the angle of inclination of the crossing fibres to be increased with consequent tendency towards widening; while in the middle of the board the loss of water takes place more equally and more or less excludes this widening tendency. (*b*) Or possibly at first during the drying the water near the ends is largely in the lumina, as well as in the walls, so that the latter do not lose much, while the evaporation draws water to the ends from the middle, which probably contains less water in the lumina and becomes depleted of this more rapidly than at the ends. Between these alternatives it is impossible to decide until further researches are conducted on shrinkage in general.

Changes of dimensions of Yang 1 during Swelling and Shrinkage.

During swelling the percentage average increases in linear dimensions were: in length ·079; in width at the ends and middle 6·98 and 7·4 respectively. Thus the percentage tangential elongation was 91 times as great as the longitudinal.

TABLE C I.

Date	Time	Weight in grammes	Difference	*yx*, *yxb*	Difference	*y*, *yb*	Difference	*y*, *yx*	Difference	*yb*, *yxb*	Difference	Middle	Difference	
VII. 7	11	1034·6	—	61·072	—	61·085	—	13·645	—	13·502	—	13·478	—	Measured, then weighed, the curves then taken, then weighed again (12 o'clock), then measured
,, ,,	12	1026·85	7·75	,, ·068	·004	,, ·076	·009	,, ·640	·005	,, ·496	·006	,, ·470	·008	
,, 8	10.40	885·1	141·75	,, ·066	·002	,, ·074	·002	,, ·582	·058	,, ·444	·052	,, ·412	·058	
,, 9	10.45	826·2	58·9	,, ·040	·026	,, ·050	·024	,, ·514	·068	,, ·378	·066	,, ·252	·160	
,, ,,	4.30	811	15·2	,, ·024	·016	,, ·042	·008	,, ·436	·078	,, ·324	·054	,, ·165	·087	
,, 12	4	752·1	58·9	,, ·028	−·004	,, ·050	−·008	,, ·058	·378	12·970	·354	12·861	·304	
,, 13	11	747·5	4·6	,, ·028	·0	,, ·052	−·002	,, ·014	·044	,, ·916	·054	,, ·839	·022	
,, 15	10.35	746*	1	,, ·032	−·004	,, ·058	−·006	12·990	·024	,, ·882	·034	,, ·830	·009	
,, 16	10.50	741·625	4·375	,, ·028	·004	,, ·057	·001	,, ·956	·034	,, ·824	·058	,, ·790	·040	
,, 19	10.30	742·4	−·775	,, ·032	−·004	,, ·056	·001	,, ·963	−·007	,, ·832	−·008	,, ·807	−·017	
XI. 29	—	726·82	15·58	,, ·032	·0	,, ·048	·008	,, ·828	·135	,, ·711	·121	,, ·672	·135	
(XII. 10)	—	(728)												
Total difference			307·28		·040		·037		·817		·791		·806	The mean between the two end contractions ·791 and ·817 is ·804, while middle contraction is ·806
Percentage of full swollen length					·065		·060		6 nearly		5·86 nearly		5·98	

* Fragment weighing ·59 fell off, hence to this and all subsequent weights ·59 must be added. July 14 much rain fell; July 15 the air was moist and weather threatening.

TABLE C II.

Date	Time	Weight in grammes	Difference	Mean length in cm. *yyb . yxyxb*	Difference	Mean end width in cm. *yyx . ybyxb*	Difference	Middle width in cm.	Difference	Length × width of ends	Decrease of area per 10 grammes of water lost, estimated by length × width, average of ends	Decrease of area per 10 grammes of water lost, estimated by length × width, average of whole board
VII. 7	11	1034·6	—	61·0785	—	13·5735	—	13·478	—	829·011	—	—
,, ,,	12	1026·85	7·75	,, ·072	·0065	,, ·568	·0055	,, ·470	·008	828·625	0·5	0·66
,, 8	10.40	885·1	141·75	,, ·070	·002	,, ·513	·055	,, ·412	·058	825·239	0·24	0·24
,, 9	10.45	826·2	58·9	,, ·045	·025	,, ·446	·067	,, ·252	·160	820·811	0·75	1·23
,, ,,	4.30	811	15·2	,, ·033	·012	,, ·380	·066	,, ·165	·087	816·621	2·75	3·2
,, 12	4	752·1	58·9	,, ·039	−·006	,, ·014	·366	12·861	·304	794·361	3·76	3·46
,, 13	11	747·5	4·6	,, ·040	−·001	12·965	·049	,, ·839	·022	791·383	6·47	4·6
,, 15	10.35	746·5	1·0	,, ·045	−·005	,, ·936	·029	,, ·830	·009	789·678	(16·95)	(10·95)
,, 16	10.50	742·125	4·375	,, ·0425	·0025	,, ·890	·046	,, ·790	·040	786·838	6·48	6·07
,, 19	10.30	742·9	−·775	,, ·044	−·0015	,, ·8975	−·0075	,, ·807	−·017	784·538	—	—
XI. 29	—	727·32	15·58	,, ·040	·004	,, ·7695	·128	,, ·672	·135	779·481	4·97	4·9
Total difference			307·28		·0385		·804		·806	49·53		
Percentage of initial maximum			·2		·063		5·92		5·98	6 (circa)		

The swelling per cent. in a tangential direction is 94 times as great as that in a longitudinal direction

During shrinkage the board did not dry completely to its original condition (it retained only about 5·5 g. more water), but making allowance for this fact, its shrinkage was not relatively (nor absolutely) so great as the swelling. The percentage average decreases in linear dimensions were: in length ·063; in width at the ends and middle 5·92 and 5·98 respectively. The percentage tangential shortening was 94 times as great as the longitudinal.

Changes in area. During absorption and emission of water it has been shown that synchronous longitudinal and tangential changes in dimensions may be opposite in sense. The question arises therefore: During absorption and emission of water does the volume or surface as a whole always respectively increase and decrease? The statistics available prove that a decrease and increase in the amount of water respectively cause corresponding decrease and increase in area: thus anomalous changes in length are overborne by the more normal changes in width (the one apparent exception recorded in Table C II July 19th is discussed later).

The subjoined Table D records the changes in surface for each ten grammes of water gained or lost. In order to show the corresponding conditions as regards the statistics concerning drying and soaking respectively the data are ranged in columns in reverse order.

Table D.

Swelling			Shrinking		
Date and hour	Increase of area per 10 grammes of water	Weight of board and water	Decrease of area per 10 grammes of water	Weight of board and water	Date and hour
VI. 21	—				
„ 22 10	4·07		4·97		XI. 29
„ „ 2.15	2·3	721–874	6·07		VII. 16
			(10·95)		„ 15
„ 23	4·06		4·6	727–826	„ 13
„ 24	3·29		3·46		„ 12
„ 25	2·2	874–896	3·2		„ 9 4.30
„ 28	1·01	896–938	1·23	826–885	„ 9 10.45
„ 29	0·05	—	—	—	—
VII. 1	0·22	967–986	0·24	885–1026	„ 8
„ 5	0·387	986–1017	0·66	1026–1034	„ 7 12
„ 7	0·13	1077–1034	—	—	—

Both sets of observations show that the greatest change in area (and, judging by the statistics concerning the radial board, in volume) induced

by gain or loss of the same amount of water takes place when the wood is poor in water, and that as the wood contains larger amounts of water there is a decline in such a change in area. This is in part due to the larger proportion of water present in the lumina of the wetter wood; moreover if we assume that the same loss of water always causes the same decrease in volume of the solid wood-substance (cell-wall) the smaller diameters of the wood-constituents in the dry phase have to be considered. But the irregularities in changes of area near the beginning and conclusion of the drying and soaking require explanation. One source of misinterpretation is brought out by the change between July 16 and 19, when there was a slight hygroscopic absorption of water and yet a decrease in area: probably this additional moisture was superficially distributed to a considerable extent, and the main mass of the wood was probably drier on the later date. During drying, small changes in weight of the board may on the one hand be associated with relatively greater changes in the distribution of moisture within the board; moreover during such small changes a slight underestimation of the amount of water lost will cause the increase in area to appear excessive. And it will be noted that during drying apparently unduly large results are associated with small changes of weight, viz. on July 7, 11 to 12 a.m., July 12–13, July 13–15, and July 15–16. On these dates the maximum result (dividend) appears when the loss of water recorded was the minimum (July 13–15). During soaking there were no such small changes in weight, and, excepting at the beginning and end, no sudden or great changes in the rate of dimensional increase. The contrast between the results between June 21 and 22 at 10 a.m., and June 22, 10 a.m. to 2.15 p.m., is possibly due to the fact that the increased water-content during the former period was due to slow absorption of aqueous vapour, and during the latter period to rapid intake of liquid water much of which would be in the lumina and in operation on swelling; but the unexplained smallness of the increase in width of the middle of the board on June 22 is also partly responsible.

Twisting of Yang 1.

The warping along the length and transversely across the ends have already been discussed; it remains to consider the warping along the diagonals and the consequent twisting.

The deviations from the straight were measured in millimetres on the curves recorded by Professor Dalby's instrument; but as the

latter doubles the deviation the number recorded in the subjoined Table E must be halved in order to give the true facts. The measurements recorded from June 21 to July 7 relate to the board during the absorption of water, those subsequent to July 17 to the drying board.

The diagonal *yx . yb* runs rather across the grain exposed at the surface and consequently underwent greater elongation and shortening than did *y . yxb*, which tends more towards parallelism with that grain. The lengths of the diagonals measured were approximately 56 centimetres.

In Table E:

Column 1 represents the time of observation.

Column 2 records the amount of water absorbed subsequent to the immediately preceding period.

Columns 3 and 7 record the nature of the curve, whether concave (*c*), or convex (*v*), or straight (*s*) of the whole board along the diagonal and the number of millimetres that the middle is sunk or raised above the true base line as recorded by the instrument.

Table E.

1	2	3	4	5	6	7	8	9	10
		yx . yb				*y . yxb*			
Date and hour (circa)	Grammes of water absorbed	Shape and deviation			Twist deviation	Shape and deviation			Twist deviation
		Whole	*yx* half	*yb* half		Whole	*y* half	*yxb* half	
VI. 21	—	*c* 16·25	11·25	12·0	54	*v* 8·75	7·75	6·75	4·75
„ 22 11	16·45	*c* 15·5	11·75	9·25	51	*v* 8·25	6·5	6·25	3·75
„ „ 3.25	59·15	*c* 14·25	6·5	12·5	53	*v* 9·75	7	6·75	6·75
„ 23 11	44·8	*c* 9·25	3·75	8·5	33·5	*v* 4·5	3	3·5	8
„ 23 3.50	1·1	*c* 9·25	4·5	8·25	37	*v* 4·375	3	3	0
„ 24 12	32	*s* 0	0	0	1	*v* ·8	·5	0	0
„ „ 2.50	−·7	*s* 0	0	0	0	*s* 0	0	0	0
„ 25 12.5	18	(*s*) 0	0	0	0	*s* 0	0	0	0
„ 28 11.20	46·6	(*v*) 0	(*v*) ·75	*s* 0	0	*s* 0	0	0	0
„ 29 12	29·2	*v* 1·5	1·5	·7	0	*s* 0	0	0	·5
VII. 1 11.35	19·3	*v* 1·5	·75	·5	·25	*s* 0	0	0	0
„ 5 11.25	30·5	*v* 2·5	2·0	1·0	·5	(*c*) ·5	·5	·1	1·0
„ 7 11.30	17·5	*c* 2·75	2·5	1·25	0	(*c*) ·5	·5	·1	1·75
„ 9 12.30	−208·4	*c* 2·75	2	2·5	0	(*c*) ·1	·375	·1	0
„ 13 12.30	−78·7	*c* 13·5	9	10·0	44				
„ 15 11.30	− 1·0	*c* 15	11·25	11·5	51				
„ 16 11.45	− 4·4	*c* 16	11·5	11·75	52				
XII. 10 12	−13·6	*c* 18·75	13·5	14·0	64				

Columns 4, 5, 8, 9 similarly record the deviation in millimetres of the middle point of each half of the respective diagonals; columns 4 and 8 denoting the half (top) towards the *y* . *yx* end, and columns 5 and 9 denoting the half (bottom) towards the *yb* . *yxb* end.

Columns 6 and 10 record the amount of twist by giving the number of millimetres that the end point is raised above the base line, as recorded by the instrument.

Table E shows that on June 21 the dry board was warped and twisted, being on one face (*y*) concave along one diagonal *yx* . *yb* and convex along the other, *y* . *yxb*. With the absorption of water these diagonals by differential elongation lost their curvature, and were almost or quite straight on June 24 and 25. As water continued to be absorbed until July 5 the original curvatures of the diagonals were gradually reversed, *yx* . *yb* becoming convex and *y* . *yxb* concave, the reversal taking place more obviously along *yx* . *yb* (crossing the grain). During the drying from July 7 onwards (and even before this, for some unknown reason, in the case of *yx* . *yb*) the curvatures gradually reverted to those originally present in the dry board.

It is worthy of note that the amounts of warp and twist were not exactly determined by the amount of water contained. This fact is demonstrated by comparisons between the conditions of the board on June 21 and on July 16 and December 10. The greater twist and warp during the final drying are probably due to the fact that the loss of water was more rapid in the warm dry air of the laboratory than during the original industrial seasoning of the board. This consideration emphasizes the special importance of carefully regulated seasoning of cross-grained woods.

Observations on Yang 2.

The board named yang 2 was cut tangentially and its faces were lettered as in the case of yang 1; on one face, *Y* and *Yx* denoting the top corners, *Yb* and *Yxb* the bottom corners; and on the other face, *y* and *yx*, *yb* and *yxb* denoting the corresponding points, but *y* was superposed on *Y*, that is to say *Y* and *y* are at the same edge of the board. Thus for instance *YYb* and *yyb* represent the same side of the board on the two different faces and *Y* . *Yxb* and *y* . *yxb* represent the same diagonal on the two faces.

Set F.

The first set of measurements, recorded in Table F, were made by means of the measuring rod during the absorption of water. The changes in length were too slight to be accurately estimated. On the other hand measurements of the diagonals and across the two ends were possible, and evidence in favour of their substantial accuracy is supplied by the fact that the widening of the board at both ends was the same on the average, viz. ·6375: moreover on the face *y* it was ·65 at each end, and on face *Y* ·625 at each end. This represents a widening of 5·3 per cent. of the full length attained. But it must be noted that when the board was first placed in water it was not sufficiently dry to be in equilibrium with the atmospheric humidity of the laboratory; this was proved in the second set of observations when the board was dried.

Table F.

		Diagonal				Transverse (tangential)			
Date	Weight	*Y . Yxb*	*Yx . Yb*	*y . yxb*	*yx . yb*	*Y . Yx*	*y . yx*	*Yb . Yxb*	*yb . yxb*
April 29	588·4	44·5	44·3	44·3	44·3	11·5	11·55	11·25	11·25
May 3	672·6	44·6	44·5	44·5	44·4	12·08	12·1	11·75	11·8
„ 5	688	44·6	44·5	44·475	44·4	12·05	12·125	11·8	11·85
„ 11	763·6	44·65	44·55	44·6	44·475	12·1	12·175	11·85	11·9
„ 17	787	44·6	44·5	44·55	44·475	12·125	12·2	11·875	11·9
Difference between Apr. 29 and May 17	—	0·1	0·2	0·25	0·175	0·625	0·65	0·625	0·65

During soaking the board twisted, but the direction of the twist was opposite to that of yang 1 in conformity with the fact that on the two faces its exposed oblique grain ran in directions representing spirals reverse in sense to those of yang 1. On face *Y*, the diagonal *Y . Yxb* ran more with the grain and therefore showed less elongation (·1) than did *Yx . Yb* (·2) which was directed more across the grain and became convex. For the same reason the elongation of *yx . yb* (·175) was less than that of *y . yxb* (·25). Consequently of the two superposed diagonals *Yx . Yb* and *yx . yb* the former became convex and the latter concave: while of the other two superposed diagonals *y . yxb* became convex and *Y . Yxb* concave: the result was the above mentioned twist. Naturally in subsequent drying these relations were reversed.

Set G.

The results of the measurements of yang 2 during its final process of drying from a saturated condition are given in Table G.

The board at first decreased in length, but thereafter continued to elongate. In this respect it differed from the tangential board yang 1, but agreed with the radial board. The final loss of length was exceedingly small, merely ·012 per cent. of the maximum length, but the maximal loss was ·03 per cent. These figures are far less than those for yang 1, and even less than for the radial board.

The shrinkage in width of the ends was 6·29 per cent. of the maximum width, and was thus greater than those of yang 1 (5·92) and the radial board (5).

When the shrinkage in area is assessed by the changes in length and in width of the ends it will be seen that the loss of width atoned for the slightness in the shortening: for the shrinkage of area was 41·266 cm.^{2}, compared with 49·53 cm.^{2} in the case of yang 1. Yang 2 was only about 48 cm. long, whereas yang 1 was about 61. Had yang 2 been of the latter length its contraction in area would have been (*circa*) 52·43 cm.^{2}, thus slightly more than that of yang 1.

The changes in length and width from May 28, 1915 to February 4, 1916, are worthy of special note as they occurred when the board was so far dry that it gained or lost water solely according as the air became moister or drier; and during this phase the weight of the board varied only between 570·075 and 570·48 g.

Radially Cut Board.

The first set of observations were made while this board was absorbing 309 g. of water. As a measuring rod was employed for the purpose the longitudinal measurements were found to be useless. As regards the transverse measurements the ultimate increase in width was 5·5 per cent. of the maximum attained. This statistic is not of value as showing the full radial expansion, for it was subsequently found that when first measured (and weighed) the board was not fully dry in relation to the laboratory air. Its original weight was 719 g., whereas at the conclusion of the second set of observations the board had dried down to a weight of 706·5 g.

During the soaking, in contrast with the tangential boards, the radial board remained flat and devoid of any twist.

TABLE G.

Date	Weight	Length in centimetres					Width in centimetres				
		Y . Yb	*Yx . Yxb*	*y . yb*	*yx . yxb*	Average	*Y . Yx*	*y . yx*	*Yb . Yxb*	*yb . yxb*	Average
May 17	779·5	48·036	47·982	48·03	47·982	48·0075	13·720	13·752	13·534	13·552	13·640
,, 18	761·3	,, ·035	,, ·982	,, ·03	,, ·982	,, ·00725	,, ·702	,, ·744	,, ·529	,, ·538	,, ·628
,, 19	659·8	,, ·020	,, ·970	,, ·014	,, ·964	47·992	,, ·672	,, ·719	,, ·482	,, ·492	,, ·591
,, 28	570·1	,, **·028**	,, ·964	,, **·016**	,, **·965**	,, **·993**	12·844	12·882	12·706	12·704	12·784
June 3	570·15	,, ·032	,, ·972	,, ·018	,, ·970	,, ·998	,, ·854	,, ·900	,, **·696**	,, **·702**	,, ·788
July 13	570·075	,, **·035**	,, **·974**	,, **·021**	,, **·972**	**48·0005**	,, ·854	,, ·900	,, ·686	,, ·696	,, ·784
Feb. 4	570·48	,, **·032**	,, ·975	,, ·024	,, ·974	,, ·00125	,, **·848**	,, ·900	,, ·688	,, **·692**	,, **·782**
Difference May 17–Feb. 4	—	·004	·007	·006	·008	·006	·872	·852	·846	·860	·858
Difference maximum	—	·016	·018	·016	·018	·015	·876	·870	·848	·860	

Percentage difference (decrease) of the maximum length: April 17 to Feb. 4, ·012; April 17 to 28, ·03.
,, ,, ,, ,, width, 6·29.
Absolute ,, ,, area, 41·266 sq. centimetres.

Set H.

In the second set of measurements, made on the radial board while this was drying, vernier callipers were employed, and the results are recorded in Table H.

During this thorough and at times rapid drying the board, in sharp contrast with the tangential boards, remained flat and untwisted.

During drying the shortening was slight, only ·025 or ·04 per cent. of the maximum length, being intermediate in this respect between the two tangential boards. Reverse changes in length took place, as during two phases elongation took place while at the same time decrease in width was marked. Especially worthy of note is the fact that elongation continued to the final period of drying (June 18 to November 29). Such synchronous elongation and narrowing took place not only during the period June 14 to 16, when the board was drying, but actually continued between June 16 and 17 during a slight absorption of atmospheric moisture. This latter fact was probably caused by the smallness of the amount of the water absorbed and its incomplete distribution through the wood.

The decrease in width of the ends was 5 per cent. of the maximum width, and thus less than in the tangential boards (5·92 and 6·28).

During the process of drying, even when the board was elongating, the area of the faces (as assessed by length, and width of the two ends) continued to decrease. The whole decrease in area was less (40·907) than in the tangential boards.

There is evidence that soaking caused the board to increase in width permanently, for the succeeding statistics show that for similar changes in water-contents shrinkage in width during drying was less than expansion in width during soaking. It must be noted in regard to these statistics, that during soaking the width measured was not the whole width of the board but the distance between two pencil points 11·325 to 12·15 cm. apart, whereas during drying the whole width of the board, 13·258 to 12·802 cm., was measured. Hence for perfectly similar comparison the statistics of widening given below are too small.

	Change of weight	Gain or loss of water	Change of width of *Y* . *Yx* and *Yb* . *Yxb*
Board absorbing water	722·1—1038 g.	315·9 g.	·6 cm.
„ losing „	1038—723·3 g.	−314·7 g.	−·5 cm.
„ absorbing „	719·1—1038 g.	318·9 g.	·655 cm.
„ losing „	1038—717·4 g.	−320·6 g.	−·577 cm.

Table H.

Date	Weight in grammes: Weight of board	Weight in grammes: Loss of water	Length in centimetres: $Y.Yb$	$Yx.Yxb$	$y.yb$	$y.yxb$	Width of ends in centimetres: $Y.Yx$	$y.yx$	$Yb.Yxb$	$yb.yxb$	Average length in centimetres: Length	Difference	Average width of ends in centimetres: Width	Difference	Average area in square centimetres (length × average end width): Area	Difference	Decrease of area per 10 c.c. of water lost
VI. 9	1038		61·052	61·042	(61·062)	61·032	13·258	13·238	13·316	(13·330)	61·047		13·285		811·009		
„ 10	891·1	146·9	„ ·050	„ ·038	„ ·060	„ ·028	„ ·230	„ ·212	„ ·288	„ ·306	„ ·044	·003	„ ·259	·026	809·382	1·627	0·11
„ 11	818	73·1	„ ·040	„ ·030	„ ·047	„ ·019	„ ·172	„ ·152	„ ·232	„ ·236	„ ·034	·010	„ ·198	·061	805·527	3·855	0·53
„ 14	732·75	85·25	„ ·016	„ ·024	„ ·032	„ ·018	12·900	12·877	12·878	12·876	„ ·0225	·012	12·883	·315	786·146	19·381	2·27
„ 15	723·3	9·45	„ **·030**	„ ·010	„ **·038**	„ **·020**	„ ·770	„ ·752	„ ·802	„ ·810	„ **·0245**	−·002	„ ·783	·100	780·070	6·076	6·43
„ 16	717·4	5·9	„ ·028	„ **·026**	„ ·037	„ ·016	„ ·710	„ ·695	„ ·750	„ ·764	„ **·0267**	−·002	„ ·730	·053	776·873	3·197	5·42
„ 17	717·7	−·3	„ ·030	„ **·024**	„ ·038	„ ·017	„ **·704**	„ **·688**	„ **·748**	„ **·764**	„ ·0272	·0005	„ **·726**	−·004	776·620	0·253	8·43
„ 18	713·8	3·9	„ ·024	„ ·024	„ ·036	„ ·010	„ ·670	„ ·650	„ ·723	„ ·728	„ ·0235	·004	„ ·693	·033	774·565	2·055	5·27
XI. 29	706·52	7·28	„ **·032**	„ **·033**	„ **·043**	„ **·020**	„ ·589	„ ·576	„ ·638	„ ·668	„ **·032**	−·009	„ ·618	·075	770·102	4·463	6·38
Difference:																	
VI. 9–XI. 29 (whole)	—	—	·020	·009	·019	·012	·699	·662	·678	·662	—	·015	—	·667	—	40·907	—
VI. 9–VI. 14	—	—	—	—	—	—	—	—	—	—	—	·0245	—	—	—	—	—

Percentage loss of the maximum length: final (June to November), 0·025.

„ „ „ „ maximum (June 9th to 14th), 0·04.

„ „ „ width: final and maximum, 5.

Finally percentage loss of width = 200 × percentage loss of length.

Figures printed in thick type mark cases where the change in dimensions is in sense contrary to the change in the amount of contained water a minus sign denotes the same.

While 323 (715·2 to 1038) g. of water were absorbed the average *measured* width of the same face decreased by ·612 cm., whereas while the board underwent more thorough drying and lost 331·5 (1038 to 706·52) g. of water, the average *whole* width decreased by ·673 cm., which represents a decrease of ·606 on the width measured during absorption.

I have pleasure in tendering thanks to Professor Dalby for his extreme kindness in inventing and providing the instrument for registering the changes in curvature, and to Mr Alexander Howard for presenting the boards for investigation.

THE DALBY PROFILE RECORDER.

By W. E. DALBY, M. Inst. C.E., F.R.S.

Professor of Engineering, City and Guilds Engineering College.

(With 7 Text-figures.)

1. Introduction.

A short time ago Professor Groom asked me if a method could be devised to enable him to measure the shape of the surface of a timber sample with reasonable speed and accuracy so that measurements taken from time to time could be compared in order to study questions relating to the warping of timber.

In response to this request I designed a machine by means of which the shape of the surface could be explored and recorded automatically without the necessity of taking a single measurement directly.

The result of the exploration of the surface by the machine is a drawing showing the shape of the surface along parallel lines spaced at definite distances apart.

Such a drawing is seen in Fig. 1. It is the result of exploring an artificially prepared surface of a piece of pine along five parallel lines. The surface was made specially irregular in order to illustrate the working of the machine.

The five datum lines numbered respectively 1 to 5 and the corresponding profile curves were drawn in 3 minutes. The datum lines are spaced 1 inch apart and the length of the record is about 2 feet. The size of the sample used in the particular machine in which the record was drawn is $30\frac{1}{2}$ inches long and 6 inches wide.

The curves on the drawing are really the profiles of five equidistant sections of the timber sample taken normally to a reference plane. The profiles recorded show the variations of shape to twice the actual size for convenience of measurement. The machine can be designed to give the record the actual size or any multiple of it.

2. The Reference Plane.

The machine is so designed that the datum lines in the record correspond to lines lying in a common plane in the timber sample.

This plane is called the PLANE OF REFERENCE. The plane of reference in the timber sample corresponding to the lines on the record is defined by any selected point on the timber surface to be explored. Having selected a suitable point the exploring roller of the apparatus is set to the point and then the plane through this point parallel to the plane defined by the surfaces of three studs to which the sample is clamped is the plane of reference from which ordinates to the surface are to be measured. The roller was set on the sample to the point corresponding to *Z* in Fig. 1.

A reference to Fig. 2 will make this clear. *A*, *B*, *C* are three supporting studs in the machine. The upper surfaces of these studs define a plane. Let a piece of timber be laid on these three points. Let *Z* be the point in the upper surface of this piece of timber to which the exploring roller is set. Then a plane through *Z* parallel to the plane defined by the points *A*, *B*, *C* is the PLANE OF REFERENCE from which all ordinates are measured to the undulating surface. In all forms of the machine there will be found three supporting studs corresponding to the points *A*, *B*, *C*. These studs may be regarded as three blunted points, the upper surfaces of which define what may be called the PLANE OF THE MACHINE.

The plane of reference is parallel to the plane of the machine at a distance from it determined by the particular point in the surface to which the roller is set at the beginning of the process of taking a record.

The traces of these planes are shown in the vertical elevation Fig. 3.

If the point *Z* is in the same plane as the points *A*, *B*, *C* then the plane of the machine and the plane of reference coincide. This condition is practically fulfilled in some

Fig. 1. Profile curves from an artificially prepared surface of a piece of pine.
In the original record the datum lines are 1 inch apart.

forms of the machine. In other forms the three points A, B, C are in the lower surface of the timber sample and the point Z is in the upper surface so that the plane of reference is above the plane of the machine by approximately the thickness of the sample.

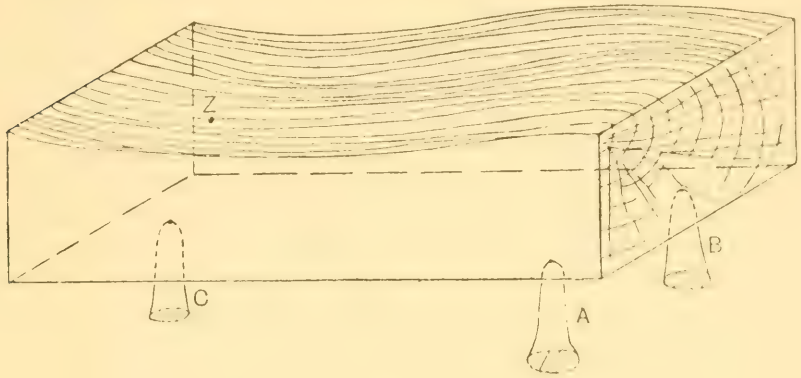

Fig. 2

It is only when the sample is clamped at three points that a true record of the surface can be made. The act of clamping at more than three points distorts the timber so that the family of profile curves correspond to a surface slightly strained, and the surface will therefore change in shape immediately the sample is removed from the machine. When however it is clamped at three points only the

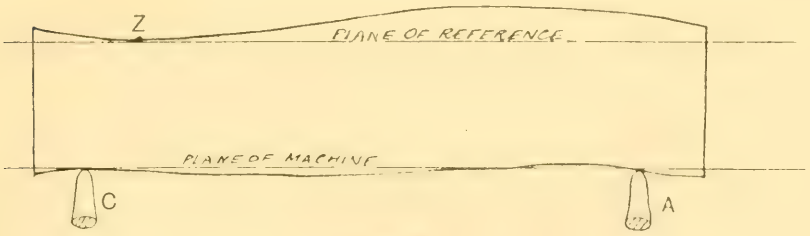

Fig 3

sample is quite unstrained, and moreover it can be removed from the machine and can be replaced in it again in the same position relatively to the frame, providing always that it is clamped at the same three points. The initial clamping points should therefore be carefully marked on the sample by ringing them round or by any other convenient method.

The position of the point Z must also be carefully marked on the surface so that the exploring roller can be re-set to this point when taking subsequent records of the surface. The re-setting to Z ensures that the families of profile curves in all the series of records which may be taken of the surface are recorded in relation to the same reference plane. In other words by re-setting the roller to Z the position of the reference plane in relation to the plane of the machine is maintained substantially without change however often the sample is replaced in the machine. In the time elapsing between two successive measurements of a family of profile curves of the surface, warping slightly changes the distances of the point Z on the surface from the clamping points; but the change is quite negligible in its influence on the position of the reference plane if the point Z is initially chosen close to a clamping point. In Fig. 1 for example, Z is taken as close to the clamp A, Fig. 4, as possible. It would probably be better in a definite series of experiments to take Z as near to the central clamping point as possible (C in Fig. 4). Then the warping of the surface would have the least effect in changing the relative positions of the reference plane through Z and the plane of the machine.

Warping and shrinking also change the relative positions of the three clamping points themselves during lapse of time. The change is likely to be slight and the effect on the relative position of the reference plane and the plane of the machine negligible. To secure uniformity in practice it is advisable to re-clamp a particular sample so that the central clamp (like C Fig. 4) grips the timber at the same point in all the re-settings of the series. The clamps A and B will then grip the sample at points displaced from the original points by amounts due to shrinkage in the linear dimension of the imaginary triangle formed by joining the three points at which the sample was originally clamped.

In cases where great accuracy is required the hole-slot-plane method of clamping may be used. Assuming the timber to be hard enough to bear clamping without appreciably indenting the surface, a conical hole is formed in the timber to receive the conical point C (Fig. 2); a V-groove pointing towards C is formed to receive the conical point B; and the surface of the timber rests on the conical point A. The timber sample when clamped down on to these conical points is then fixed relatively to the frame of the machine in the most accurate manner possible.

In cases where the timber is soft and therefore the conical supporting points are likely to form indentations in the surface, metal

screws may be screwed into the timber, the heads of these screws being specially formed, the screw at C with a conical hole, the screw at B with a V-slot, and the screw at A with a plane head.

The reference plane will stand at a fixed distance from the plane of the machine defined by the hole-slot-and-plane clamping except for the negligibly small error produced by warping and shrinking in the distances of Z from the clamping points. This error is minimised by selecting Z as close to the conical hole as possible.

3. General Description of a Recorder.

The particular form which the profile recorder takes depends upon the purpose for which it is required and upon the sizes of the samples which are to be tested.

Apparatus may be designed for drawing the profile curves of the largest planks or for studying samples of moderate size or for measuring the shape of the blades of an aircraft propeller.

One type (Mark 2) is shown by the photographs Figs. 4 and 5. This apparatus takes samples $30\frac{1}{2}''$ by $6''$ and any thickness up to $1\frac{1}{2}''$. Variations in the shape of the surface are shown twice the actual size in the record. This multiplication of the surface variations can be carried to any extent desired but the scale once settled remains constant for any particular machine.

Referring to Fig. 4 the timber sample T is clamped down by the clamps A, B and C to the corresponding studs below it. The drawing paper on which the records are to be taken is pinned down to the drawing board at D. The angle iron framework is self-contained and supports on the one side the timber sample and on the other side the drawing board.

Lying on the bottom bars of the framework is a guide frame GG consisting of two parallel rods secured in end pieces. It will be seen that this frame can be lifted from its position and can then be placed in another position defined by any one of four pairs of V-notches cut in the lower bars of the framework. These notches are pitched 1 inch apart.

The stock of the machine is supported by the guide frame. It is shown separately in Fig. 5. Its base is made of cast iron and it is grooved to slide along the guide bars GG seen in Fig. 4. It is pushed along them by hand when a profile curve is being drawn.

The stock carries an exploring roller R on the end of an arm and a

pencil *P* on the end of another arm. Mechanism connects the two arms so that the vertical movement of *R* is changed into a horizontal movement of the pencil in a direction at right angles to the direction of motion of the stock.

To draw a profile curve the stock is placed on the guide frame *GG* and the mechanism is locked in a zero position by turning the milled head *L* seen at the lower part of Fig. 5. The roller is clamped clear of the surface. The pencil is then lowered on to the paper and the stock

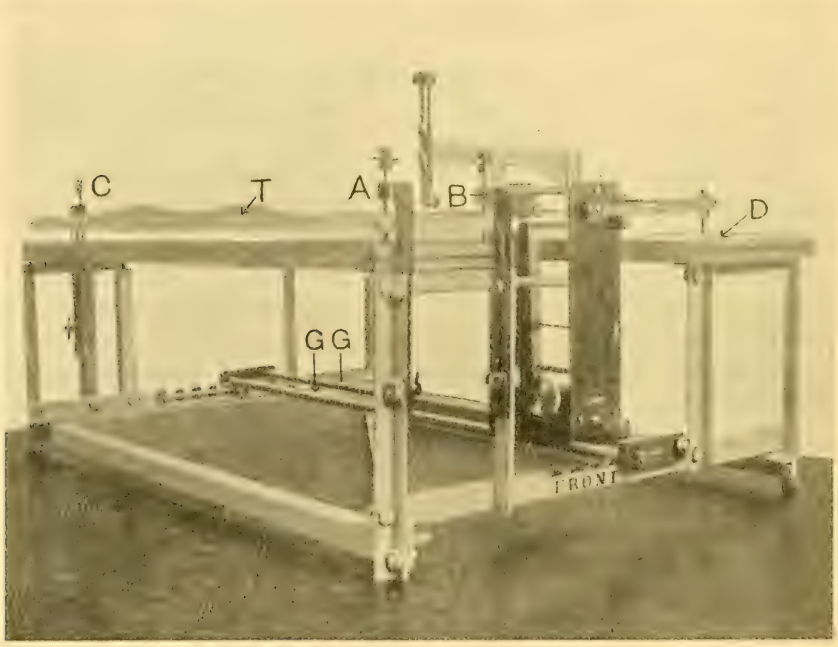

Fig. 4

is pushed along the guide rods by force applied to the handle *U*. The pencil then draws a straight datum line. The roller is then lowered into contact with the timber surface at a point of the path nearest to a clamping point and this point is marked *Z*. This point defines the position of the plane of reference, and the datum line already drawn lies in it. The lock is then released by turning the head *L* and the stock is drawn or pushed along the guide bars. The roller now follows the unevenness of the surface along the path which it is compelled to follow and the pencil *P* draws automatically the curve giving the shape of

this path twice full size. The curve is the profile of a section taken through the roller path at right angles to the plane of reference.

The process of drawing a family of curves is similar. The datum lines are first drawn one for each position of the guide frame in its

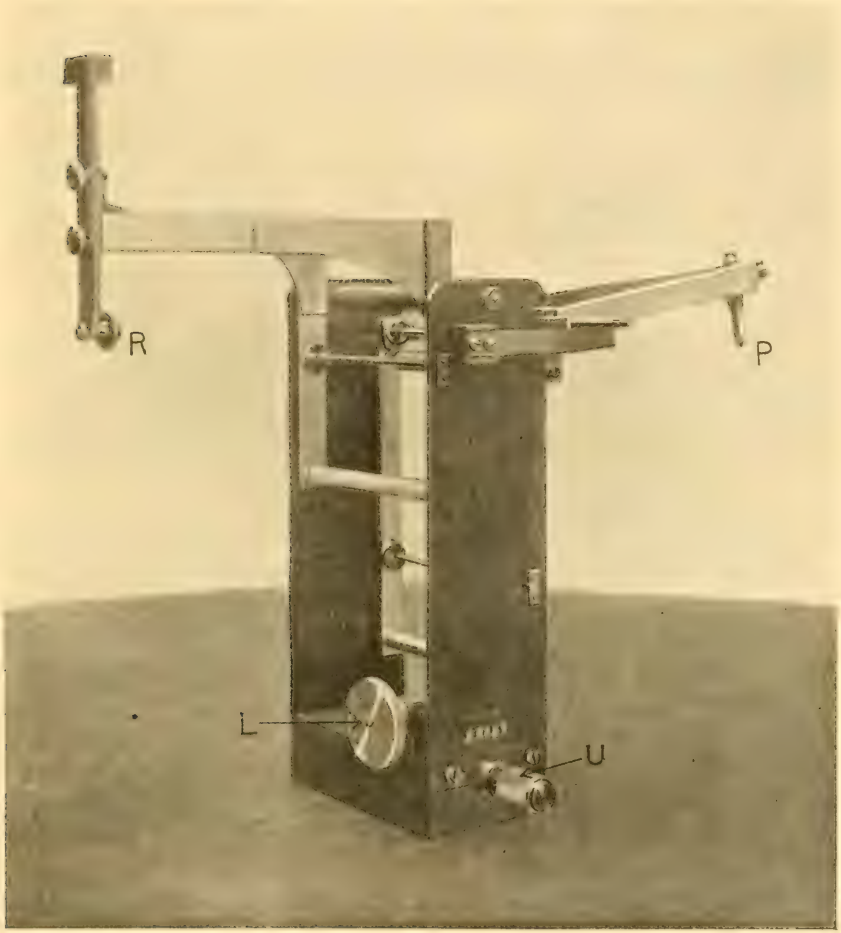

Fig. 5.

notches. Then the point Z is selected. Guide frame and stock are adjusted to bring the roller over the selected point and then the roller is lowered into contact with the surface and the roller post is securely

clamped. The profile curve corresponding to each pair of notches provided is then drawn.

The family of curves, Fig. 1, were drawn in this way.

4. Interpretation of the Profile Curves on the Record.

The datum lines lie in one plane, the plane of reference. The distance from any point on any one of the profile curves to its datum line is equal to twice the distance of the corresponding point in the timber surface to the reference plane defined by the point *Z*.

For example the point Y_1 on the Record No. 1 (drawn again in Fig. 6) is $\frac{1}{4}''$ above the reference plane containing the point *Z* on the surface of the sample. Similarly the point Y_2 is 0·2″ below this reference plane.

All the points on the timber surface corresponding with points of intersection of the profile curves with their respective datum lines lie in the reference plane containing *Z*.

For example the points on the surface corresponding to points *Zzzzz* and *yyyyy* in the record all lie in the plane of reference. The lines joining these points are contour lines in the reference plane.

Points on the profile curves at equal distance from their respective datum lines can be located. Curves through these points are contour curves for the particular distance located.

The family of profile curves drawn by the machine can therefore be used to find the contour lines of the surface explored. And these contour lines can be drawn on the record.

A family of profile curves of sections at right angles to those drawn automatically by the machine can be deduced. Suppose for example that the profile is required across the section *SS* on the record shown in Fig. 6. At each of the intersections of *SS* with the five datum lines of the record, set up (or down) the intercepts on *SS*, cut off by the respective datum lines and the corresponding profile curves. $P_1P_2P_3P_4P_5$ are points obtained in this way and the curve through them is the profile curve across the section *SS*.

Profile curves for a series of transverse sections at any assigned interval apart can be deduced from the family of profile curves drawn by the machine. Such a family is shown in Fig. 7 for transverse sections taken about 3″ apart on record No. 2, a record taken from an artificially prepared surface of a sample of pine.

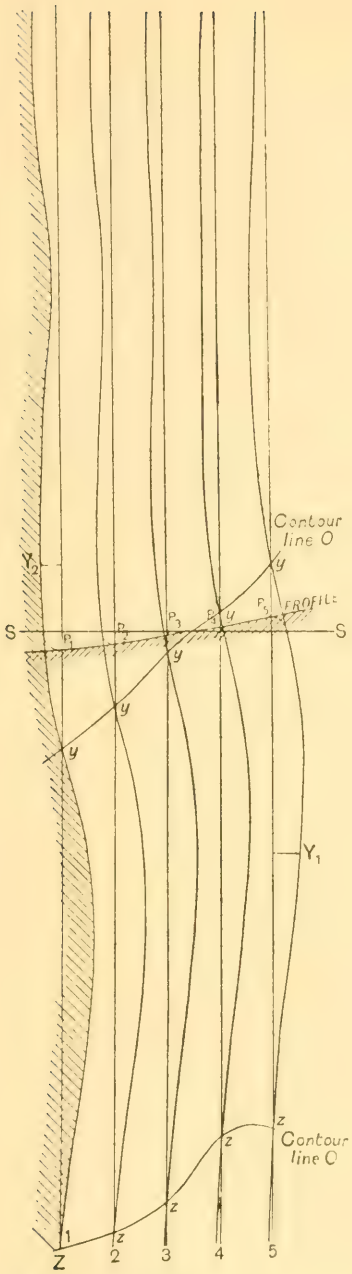

Fig. 6. PROFILE CURVES. Redrawn from Fig. 1.

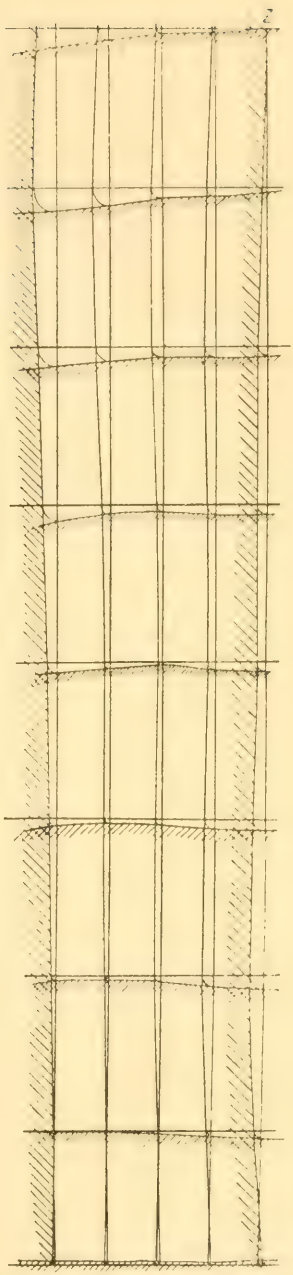

Fig. 7. Showing a family of profile curves taken from an artificially prepared surface of a pine sample together with a family of transverse profile curves deduced from them.

Each of these diagrams is a little larger than $\frac{1}{4}$ full size. In the original records the datum lines are 1 inch apart.

5. Use of the Record to plot a Contour Line on the Timber Surface.

The point on the timber surface corresponding to any point on the record can be identified by placing guide frame and stock so that the pencil is brought over the point on the record. The roller will then mark the corresponding point on the timber surface.

The series of points lying on a contour line on the record can thus be identified on the timber surface and an actual contour line can then be drawn on the timber surface itself. The meaning of such a line is that all points on it are equally distant from the reference plane containing the point Z and parallel to the points A, B and C which have been definitely imprinted on the timber surface by the three studs against which the timber sample was clamped.

THE ACTION OF ENCHYTRAEID WORMS

By the Rev. HILDERIC FRIEND, F.R.M.S.

The following paper is based on the Report which was presented to the Board of Agriculture as the result of experiments carried out in 1914–15 under the direction of Professor Gamble, F.R.S., at the Birmingham University.

The main objects were twofold:

1. To determine the question of the injurious action of Enchytraeid worms on living plants.

A long series of experiments was conducted at the Edgbaston Botanic Gardens with Asters, Antirrhinums and other plants, with a view to ascertaining what effect, if any, was produced upon the living plants by the presence of these worms, which had frequently been charged with causing decay and death. Control plants were used, and infections were made with various species of worms under a great variety of conditions. The results showed conclusively that so long as the plants are healthy and vigorous Enchytraeids do not attack them. On the other hand, when living plants begin to decline from any cause it is the usual thing for white worms to take up the work of destruction and play the part of scavengers.

While the experiments were in progress visits were paid to Droitwich, Kenilworth, Shrewsbury, Edgbaston and other places in which gardens were reported to be suffering from injurious Enchytraeid attacks. In no instance was the evidence sufficient to justify the opinion that white worms were the original aggressors, though in several instances they were busily engaged in clearing away the decaying plants. The initial injury was due to a variety of causes, such as fungi, bacteria, Julus and other pests.

2. To determine the rôle of the red-blooded Enchytraeids.

During the year advantage was taken of many opportunities to study both in the field and in the experiment house, the rôle of that group of minute worms belonging to the Enchytraeids which are characterized by red blood. They are found in moist places among vegetable debris, in sewage works, ponds, ditches, farmyards and especially by the seaside, and are found to be invariably engaged as beneficent scavengers. Though not infrequently met with in tap water their

presence is never occasion for alarm, and even if they were inadvertently swallowed no injurious effects need be feared. A report will appear in *Science Progress* for July.

The following details of observations and experiments have been selected from a large number which have been carried out, as they tend to the elucidation of the foregoing report.

1. *Antirrhinum Cultures.* (1) The object was to determine whether or not Enchytraeid worms caused failure and death.

In December 1914, twelve plants were purchased from Messrs Simpson, Florists, such as they were rearing for market. Six plants were retained for control. The other six were inoculated with Enchytraeids which had been found associated with sickly plants and were suspected of being inimical. The plants were kept in an Experiment House at Edgbaston Botanic Gardens, and regularly watched and examined. For a time the inoculated plants looked paler than the controls, but eventually they showed themselves to be stronger. All plants survived. In April 1915, the six plants were again inoculated. Blossoms perfected at the same time on both sets of plants. On May 1, the pots containing inoculated plants were examined, and found to contain many worms, but the roots were all healthy. Plants were then transferred to the Garden, and all treated alike with Enchytraeids, but not one succumbed.

2. *Antirrhinum Cultures.* (2) It was reported that Antirrhinums were suffering from root worms at Droitwich. A visit was paid to the Experimental Gardens, the plants inspected, and specimens brought away with soil. The soil contained many Enchytraeids, but was also full of Julus larvae and other suspects. These were removed, and the plants repotted with all the Enchytraeids as found. One plant died, the evidence showing that it was too weak to survive removal. The others rallied and became very vigorous in spite of the worms.

Slips taken from both these sets of Antirrhinum took root readily and made healthy plants.

3. *Antirrhinum pests.* While these cultures and experiments were in progress many plants which were diseased at Edgbaston, Kenilworth and elsewhere were examined. In the case of seedlings, forced in frames and greenhouses, fungoid growths were the chief cause of failure. Out-of-door plants suffered from various causes, but in no instance could evidence be found for convicting Enchytraeids.

4. *Aster Cultures.* December 2, 1914, Messrs Sutton and Sons, Reading, kindly supplied three packets of seed for experiment. These were *A. sinensis* (type) with the varieties *Victoria* and *Ostrich Plume.* The Head Gardener at Edgbaston had ceased to grow Asters as they had of recent years proved a complete failure.

The foreman sowed seeds of each kind (which were marked 1, 2 and 3) in the soil used in the gardens used for bringing on seedlings, and these were allowed to grow till they were ready for repotting. While a number of plants were reserved in the original large pots for control, others were planted in (1) loam, (2) sterilized leaf mould, and (3) Enchytraeus infected soil and manure. They grew side by side and flourished.

Next, some of the repotted plants were inoculated with Enchytraeids while the rest were retained as controls, but not a single plant fell a victim to the treatment.

A second set of experiments yielded the same results. Seeds of the same samples were sown in pots containing (1) loam, (2) sterilized leaf mould, (3) Enchytraeus infected soil and (4) sand. These in due course were infected with Enchytraeids, including species found in the rubbish heaps in the Botanical Gardens; and again, all survived.

Some of the plants were transferred to the open border, and others planted in soil containing Enchytraeids, but they made the most perfect growth.

5. *Miscellaneous investigations.* During the year, every opportunity which presented itself for investigating plant diseases was utilized. Turnips near Coventry, Lilies at Shrewsbury, Peas at Erdington, bowling green Grass on the Solway, and fairy rings at Cheltenham were carefully examined, and all the evidence for and against the Enchytraeids carefully weighed. In no single instance could it be shown that white worms were the aggressors, but there were frequent evidences that Enchytraeids came in as secondary agents when plants were already weakened, and so played the part of scavengers.

Conclusion. Enchytraeid worms are abundant in leaf mould, and are often found associated with decaying field and garden crops on which account they have been suspected of causing their decay and death. The evidence shows that they are a beneficent group of animals of great economic value to the forester, gardener and farmer. They break up decaying matter and prepare it for further service but it remains to be shown that in any case they are the direct cause of the diseases of plants.

THE FOOD OF SLUGS AND THE DEVELOPMENT OF ANOPLOCEPHALIDAE

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I have just read, a little tardily, but with much interest, the note of Miss Marie V. Lebour, "Some feeding habits of slugs," published in *The Annals of Applied Biology*, Vol. 1, Nos. 3–4, January 1915, p. 393.

Miss Lebour has discovered that the slugs, *Agriolimax agrestis* and *Arion circumscriptus*, ingest very greedily the proglottids of two Anoplocephalidae,—*Moniezia expansa* of sheep and *Cittotaenia pectinata* of rabbits; she has ascertained that the eggs of these Cestodes pass through the intestine of the Molluscs without undergoing alteration, the six-hooked embryos remaining alive; but she has not discovered any trace of the larval Cestodes in the body of the slug.

It may not perhaps be without interest to record, in this connection, the results of an experiment, very imperfect, but nevertheless important, which I had occasion to make twenty years ago and about which I have just found certain short notes.

On November 4, 1892, I collected from the faeces of a carrier pigeon coming from Neufchâtel-en-Bray (Normandy) some proglottids of *Bertiella delafondi*; these proglottids, macerated in a little water, were placed on a cabbage leaf with four grey slugs (*Agriolimax agrestis*). The next morning, I noticed that almost the whole of the contaminated leaf had been eaten.

On the 14th of November, I dissected one of these slugs, but could only find some Nematodes and Trematodes.

On the 8th and 30th of November, I repeated this operation with chicory leaves and a second lot of six grey slugs. On the 14th of December, I fed two pigeons on these slugs, each receiving three. Towards the middle of January, one of these pigeons began to evacuate proglottides of *Bertiella delafondi*.

As can be seen, this experiment has been left unfinished, since I have not observed in slugs the larval form of the parasite, and unfortunately I have not had an opportunity of repeating the experiment.

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A BACTERIAL SPOT OF CITRUS.

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(With Plates III—XIII).

IN October 1914, toward the close of the Citrus season, a grower in the Western Province submitted for examination some lemons badly disfigured by dark-coloured sunken spots; he stated that he had first noticed this blemish two seasons back, but that it was rapidly on the increase; as much as 30 per cent. of his lemons were affected, and the trouble was spreading to the Washington Navel oranges.

No fungus mycelium could be found in the discoloured tissues, but there were pockets of disintegrated cells filled with innumerable bacteria. A pure culture was obtained at once from these tissues, and a few successful inoculations carried out. It was too late in the season however to enter on a complete investigation of the trouble, and further work in connection with it had to be deferred until the present year, when the matter was taken up as soon as favourable weather conditions started the spread of infection to the new crop of fruit.

A detailed study of the causal organism has now been carried out, and a survey made of orchards in the infected area with a view to preventing if possible a further spread of infection.

LITERATURE.

Two bacterial diseases of citrus have been described, both occurring in America; the so-called "citrus canker" of Florida need not be more than mentioned here; the macroscopic characters of the "canker" are entirely different from those of the spot under consideration, it is characterised by a proliferation and subsequent suberisation of the cells of the affected tissues(4).

A much more closely related trouble however is reported from the lemon growing sections of Southern California(6); it has been

appearing occasionally for the past three years, has gradually increased and is now assuming some economic importance. This disease has been named "Black Pit of Lemon" a term which aptly describes its effects on the fruit, where it causes circular-oval discoloured spots 5 to 20 mm. in diameter, the tissue being depressed somewhat below the bottom of the normal oil glands into the white portion of the rind.

The cause of the disease proved to be an actively motile rod with a single polar flagellum, and it has been named *Bacterium citriputeale.* The description and illustrations of fruit attacked by this organism correspond very closely with those of spotted lemons from the Western Province, and it was surmised that the same organism would prove to be the source of the trouble. A thorough investigation however has shown that the organism causing the disease in South Africa is quite distinct both in morphology and in cultural characters.

Distribution.

The Botanical Division of the Agricultural Department has no officer stationed within easy reach of the citrus orchards of the Western Province; arrangements were therefore made with the co-operation of Mr C. W. Mally, the Cape Entomologist, to systematically inspect the citrus trees in the districts with a winter rainfall, but unfortunately the exceptionally rainy season prevented this plan from being carried out with any degree of thoroughness. A number of orchards however, have been inspected and a large number of specimens of suspected fruit sent to Pretoria for examination; and a certain amount of information has been gleaned as a result of circularising the principal citrus growers of the Union.

The disease is now definitely known to occur on a number of farms in the region of Simondium and Lower Paarl. It extends along the Berg River Valley and has been found occurring at Wellington, Sir Lowry Pass and French Hoek. It has also been reported as occurring at Stellenbosch, but no specimens of supposedly infected fruit from that locality have been examined.

So far as can be ascertained the Transvaal is entirely free from the trouble; there is certainly no sign of it at Warmbaths or Nelspruit, and growers at Barberton state that during a careful examination of their trees they failed to find anything at all resembling the bacterial spot. The Rustenburg area also seems to be clean; one box of oranges and lemons sent from there in response to enquiries proved

to be attacked by anthracnose (*Colletotrichum gloeosporioides*) which causes discoloured spots on the fruit not very widely differing in appearance from those caused by the bacillus. They are different in colour however, not conspicuously sunken, and can be readily distinguished when the pinkish spore pustules begin to develop.

During November and December of this year (1915), after this article was practically completed, Mr Pole Evans, the Chief of this Division, made a personal inspection of the orchards in the infected areas. At that time there was little or no fruit on the trees so that his inspection resolved itself into a search for the disease on branches of trees and nursery stock.

Branch infections were very plentiful in the orchard at Simondium where the disease was first detected, in an orchard near Cillie's Siding, and one in Zuider Paarl: at Simondium the organism attacks stems $\frac{1}{2}$ to 1 inch in diameter. At the Elsenburg Agricultural College, the nursery stock has been severely attacked but no signs of the trouble were found in any of the other nurseries which were inspected.

He saw no evidence in the orchards of the Clanwilliam, Piquetburg or Montagu Districts. It is apparently confined at present to the valleys of the Groot and Klein Drakenstein and the Berg River Valley; in this area the fruit has been attacked on a number of farms, but branch infections have only been detected in the localities mentioned above.

From field observations it would appear that the leaf is attacked first and that the disease is then communicated to the stem through the leaf stalk. As soon as the petiole and neighbouring stem tissues are invaded the leaf falls, and this would account for the fact that very few leaf infections were found on branches sent for examination.

Signs of the Disease.

The disease has been found occurring in nature on lemons of the Mediterranean type, navel oranges and naartjes, the term "naartje" including both Mandarins and Tangerines. It has been induced by artificial inoculation in lemon, orange (three varieties), naartje, shaddock, grape fruit, citron and sour and sweet limes.

In describing the appearance of affected parts, and the cultural characters of the organism, the colours named have been compared as accurately as possible with those in Ridgway's *Color Standards and Nomenclature* (5).

It was first noticed in Geneva lemons, on which it forms discoloured sunken spots varying from 1 to 3 mm. in diameter: more frequently

they are 5—10 mm. in diameter and depressed 1—2 mm. below the surface of the healthy rind. The spots are more or less round, but when a number occur in close proximity to one another, they coalesce and result in the formation of large irregular blotches (Plates III and IV).

A close examination of the spot usually reveals a small wound in the centre which has been the starting point of infection; in a few cases a large diseased area showed numerous scratches, possibly the result of swinging against a thorn in the high winds.

Young infections vary considerably in colour; one spot, 28 mm. in diameter, was maize yellow, the centre buckthorn brown, others were chamois with margin and centre of Mikado brown. In a few cases there were one or more concentric rings of brown on a lighter ground. They become much darker with age, varying in colour from Argus brown through light seal brown to blackish brown; frequently the dark central portion is surrounded by a reddish rim.

It is not a soft rot, and the spots are either leathery in texture or else quite hard. A number of affected lemons were kept for some days in a moist chamber and at the end of that time, dirty yellowish drops of a viscid substance were seen oozing from the point of infection. A similar circumstance was observed in lemons artificially infected and kept in a moist chamber.

By cutting through the discoloured spots it can be seen that there is a very definite line between the sound and the diseased tissues, in some cases only about half the thickness of the rind is affected; in others the organism penetrates right through the rind and invades the pulp. The pulp below the discoloured rind becomes rather dry looking and has a peculiar taste and smell; in severe cases it is discoloured to a cinnamon brown, only the outer parts are affected and the disease does not appear to spread in a tangential direction.

On oranges the spots are slightly different in appearance; they average 20—30 mm. in diameter and are not so deeply sunken as those on the lemons; the colour is usually buckthorn brown to Dresden brown; I have never observed spots on oranges with the conspicuous almost black discoloration so common in lemons (Plate VIII).

Naartjes become very badly infected: a number of naartjes sent direct from the orchard shewed as many as 20—30 spots of varying size on a single fruit, they were round to irregular and 1—25 mm. in diameter; slightly sunken and varying in colour from something between Mikado and Sayal brown to blackish brown (Plate VII). In some large spots with an evident thorn prick in the centre the disease

had gone right through the skin and penetrated into the pulp. The latter was not noticeably discoloured but looked rather dry and had a peculiar taste and odour.

On shaddocks by artificial inoculation a number of small spots were produced not exceeding 8 mm. in diameter; they were very slightly sunken, and cinnamon brown rufous in colour. On cutting through these spots it was found that the disease had not penetrated far into the rind; the tissues were discoloured to a dirty brownish yellow to a depth of 1·5 mm., *i.e.*, just below the oil ducts. This discoloured portion was bounded by a thin scarlet line which in some cases marked the boundary between healthy and diseased tissues. The red line is not always present, and when present is not always continuous. The red colouring matter disappeared at once when pieces of the rind were fixed in hot acetic alcohol.

Grape fruit artificially inoculated developed infected areas not unlike those on the lemons. They were sunken, rather irregular in outline, up to 1 cm. diameter, cinnamon colour in the centre with a darker rim, of Mikado or Sayal brown. Sour and sweet limes are also affected in a similar way to the lemons.

On citrons inoculation with a pure culture resulted in the formation of numerous reddened areas up to 12 mm. diameter, some of these were slightly sunken; the colour was at first zinc orange to apricot orange, and later became cinnamon brown.

The only variety of citrus fruit which has so far proved resistant is the Seville orange, which has not been found infected naturally, and which has up to the present resisted infection by artificial means.

Affected fruit cannot be kept for any length of time; the discoloured spots readily serve as a starting point for numerous fungus infections and these complete the work of destruction. Almost every fruit infected with the spot disease, if kept for any length of time, was completely destroyed by *Penicillium spp.* or, less frequently, by *Colletotrichum gloeosporioides*.

The disease not only disfigures and destroys the fruit, but affects the branches to a considerable extent. The organism seems almost invariably to attack the stems just round the leaf bases; an area of about 6—10 mm. diameter becomes water-soaked in appearance, the petiole becomes involved and sometimes the basal part of the leaf, and the leaf falls. The infection then spreads to the branch in the leaf axil, surrounds it at the base and it withers and dries up. The infected area on the main part of the stem becomes up to 20 mm. long, and

goes half way round the stem; after a time the central part loses its water-soaked appearance and turns brown (chestnut brown to olive brown) and becomes slightly sunken (Plate IX, *a* and *b*). The discoloration spreads out to the edges of the infected area which ceases to increase in size. In transverse sections the discoloration does not appear to extend into the wood, but if the bark be peeled off the surface of the woody cylinder appears slightly yellow.

Towards the end of the season, *i.e.*, in the spring, a considerable amount of gummosis may be observed from the affected stem tissues, the gum oozing very largely from the severed leaf bases. In a number of affected branches received at this time of the year the leaf subtending the flowering shoot had been destroyed, and it was a question whether the whole inflorescence would not have dropped before any fruit was set.

If the disease were confined to the fruit it would hardly be expected that it would cause any considerable damage in the districts with a summer rainfall and a dry winter, but the fact of its attacking the branches and causing the destruction of the leaves causes the trees to present a defoliated appearance towards the end of the season. It may be anticipated therefore, that even in a climate where the fruit ripened during the dry season considerable damage would be done to the trees during the summer, and their vitality considerably lowered. An exceptional winter rain such as fell in the Transvaal during last July would be sufficient to start the spread of infection amongst the fruit.

Leaf infections are comparatively rare, occasionally a small discoloured area develops in the leaf tissues, a thorn prick or small wound serving as the starting point of infection. The dark brown spot is surrounded by a yellowish zone and the injured tissues eventually fall out leaving a hole. (Plate IX, *c*).

Inoculation Experiments.

An organism was plated out from lemons received from Simondium in November, 1914; with a pure culture of this a number of lemons were inoculated by needle pricks, and in four to five days these showed a number of small sunken dark-coloured spots. Owing to the pressure of work and the fact that the lemon season was nearly over, the work was discontinued, and was not resumed until July, 1915.

At the beginning of this month the disease again became very active, shortly after the first steady rains in the Western Province, and specimens of infected fruit (both lemons and navel oranges) were

received from the same locality as before. From these a pure culture was readily obtained, and a yellowish bacillus was isolated. With these cultures an infection experiment was carried out; sound lemons obtained from Warmbaths, where the disease does not occur, were dipped in 1:1000 mercuric chloride and then washed in running water. They were then divided into four lots, each lot placed in a large glass jar with a drop-on lid, and treated as follows: in jar (1) the lemons were pricked with a sterile needle; the fruit in (2) was lightly pricked with a very fine needle then atomised with a suspension of an agar culture of the bacillus; in (3) the lemons were inoculated by needle pricks, and in (4) were injected with a hypodermic syringe. At first the lemons were kept at room temperature which even in the day time was only 13° C. After 48 hours there were slight indications of infection, the tissues in the immediate neighbourhood of the needle pricks were slightly discoloured.

After four days infections were quite marked, the oil glands in particular were discoloured, and the tissues in the vicinity of the wound were becoming slightly depressed. The fruit was then removed to the incubator at 25° C. the spots subsequently developed much more rapidly and after ten days all the inoculated fruit showed well developed infections. The fruit in the four jars may be described as follows:

(1) Control, pricked with sterile needle, quite sound, wounds caused by needle pricks barely visible on close inspection. (Plate V, *a*, right hand figure.)

(2) Pricked, then atomised with suspension of culture. There was an infection for almost every needle prick. As many as 100 spots on one lemon, the colour of the spots cinnamon buff to snuff brown and mummy brown; the diseased tissue sunken, and the spots small. (Plate V, *a* and *b*.)

(3) Inoculated by needle pricks, spots up to 6—8 mm. diameter, colour as in (2), from 20—65 sunken spots on each lemon. There was a viscid drop of dirty yellowish substance oozing from the centre of each spot; on examination this proved to be composed of capsuled bacteria, and on being plated out, yielded a pure culture of the organism with which the fruit had been inoculated. (Plate VI, *a*.)

(4) Suspension of culture in distilled water injected with hypodermic syringe; spots varied from 3 mm. to 30 mm. diameter; they were sunk 1—1½ mm. below the surface, 4—32 spots on each fruit, some of which did not appear to be in connection with a wound. The discoloration varied from Sayal brown to mummy brown. Viscid

drops of bacterial slime were also oozing from the centre of these spots. (Plate VI, *b*.)

An infection experiment on a larger scale was carried out with a quantity of citrus fruit obtained from the Government Orchard at Warmbaths; the fruit was picked from the trees in the writer's presence, taken direct to the Laboratory in boxes and the inoculations carried out two days later. There was no trace of the disease on any of the fruit in the orchard.

The cultures used were from four different sources: from natural infections on lemon, on orange and on naartje and from an artificial infection on lemon. In the schedule the last is distinguished as lemon II, the first as lemon I. In each case young cultures on nutrient agar were employed for needle prick inoculations, and young cultures suspended in sterile distilled water for atomising.

All controls were pricked with a sterile needle; inoculations were carried out in four different ways, some were atomised with a "glaseptic" sprayer without any previous wounding, others were lightly pricked with a very fine steel needle and then atomised, into a third lot of fruit the organism was introduced by means of needle pricks, and the bacillus was injected into a fourth set by means of a hypodermic syringe.

No. of Fruit	Kind of Fruit	Source of Culture	Method of Inoculation	Result
6	Ripe lemons	—	Sterile needle	No infection
7	„ „	Lemon I	Pricked then atomised	Positive
8	Green „	„ I	„ „ „ ...	„
7	Ripe „	„ I	Needle pricks	„
8	Green „	„ I	„ „	„
7	Ripe „	„ I	Hypodermic syringe ...	„
7	Green „	„ I	„ „ ...	„
5	Ripe „	—	Sterile needle	No infection
5	Ripe „	Orange	Pricked then atomised	Positive
5	Green „	„	„ „ „	„
6	Ripe „	„	Needle pricks	„
5	Green „	„	„ „	„
7	Ripe „	„	Hypodermic syringe ...	„
7	Green „	„	„ „ ...	„
5	Navel oranges	—	Sterile needle	No infection
5	„ „	Lemon I	Pricked then atomised	Positive
6	„ „	Orange I	„ „ „	„
5	„ „	Lemon I	Needle pricks	„
5	„ „	Orange	„ „	„
5	„ „	Lemon I	Hypodermic syringe ...	„
5	„ „	Orange	„ „ ...	„
8	Valencia late oranges	—	Sterile needle	No infection

No. of Fruit	Kind of Fruit	Source of Culture	Method of Inoculation	Result
15	Valencia late oranges	Lemon I	Needle pricks	Positive
8	,, ,, ,, ...	Orange I	,, ,, ...	,,
10	Seedling oranges (Parson Brown)	—	Sterile needle	No infection
11	,, ,, ,, ,,	Lemon I	Needle pricks	Positive
10	,, ,, ,, ,,	Orange	,, ,, ...	,,
3	Seville oranges	—	Sterile needle	No infection
3	,, ,, ...	Orange	Needle pricks	Doubtful
4	,, ,, ...	Lemon I	,, ,, ...	,,
3	,, ,, ...	Orange	Pricked then atomised	,,
1	Citron	—	Sterile needle	No infection
1	,, ...	Lemon II	Needle pricks	Positive
1	,, ...	Orange	,, ,, ...	,,
1	,, ...	Lemon II	Pricked then atomised	,,
1	,, ...	,, II	Hypodermic syringe ...	,,
8	Naartjes	,, II	Atomised without wound	Negative
4	Navel oranges	Orange	,, ,, ,,	,,
4	Sweet limes	Lemon II	,, ,, ,,	,,
4	Naartjes	—	Sterile needle	No infection
5	,, ...	Lemon II	Needle pricks	Positive
5	,, ...	Orange	,, ,, ...	,,
6	Sour limes	—	Sterile needle	No infection
6	,, ,, ...	Lemon II	Needle pricks	Positive
6	,, ,, ...	Orange	,, ,, ...	,,
7	,, ,, ...	Lemon II	Pricked then atomised	,,
5	Navelencia oranges	,, II	Atomised without wound	Negative
4	Sweet limes	,, II	Needle pricks	Positive
4	,, ,, ...	Orange	,, ,, ...	,,
5	,, ,, ...	Lemon II	Pricked then atomised	,,
4	,, ,, ...	Orange	,, ,, ,,	,,
5	,, ,, ...	Lemon II	Hypodermic syringe ...	,,
7	Sour limes	—	Sterile needle	No infection
5	,, ,, ...	Lemon II	Atomised without wound	Negative
5	,, ,, ...	Orange	,, ,, ,,	,,
5	,, ,, ...	Lemon II	Hypodermic syringe ...	Positive
12	Lemons	,, II	Atomised without wound	Negative
1	Shaddock	—	Sterile needle	No infection
1	,, ...	Lemon II	Needle pricks	Positive
1	,, ...	Orange	,, ,, ...	,,
4	Grape fruit	—	Sterile needle	No infection
4	,, ,, ...	Lemon II	Needle pricks	Positive
4	,, ,, ...	Orange	,, ,, ...	,,
1	,, ,, ...	Lemon II	Hypodermic syringe ...	,,
1	,, ,, ...	Orange	,, ,, ...	,,
16	Rough lemons	Naartje	Needle pricks	,,

It will be observed from the above schedule that with the exception of the Seville oranges all inoculations were positive on fruit which had

been pricked or otherwise slightly wounded. None of the lemons or other citrus fruit which were atomised without wounding developed any spots, but I do not regard this as conclusive evidence that the organism cannot find its way through the stomata, for several reasons. In the first place, the fruit which was used for the experiment had been hanging on the trees in a very dry atmosphere, and the outer tissues of the rind had become comparatively dry and leathery, and it was noticed that even after wounding these infected less readily than those used in the preliminary experiment which were cut after an exceptional spell of rainy weather; also in the first experiment a number of infections appeared on lemons which were injected with a hypodermic syringe at points where no injections had been made, and on naturally infected fruits on many spots where no wound can be found, and sections through the tissues show the bacteria in the substomatal cavity, but this will be alluded to later.

Further inoculations will be necessary before anything conclusive can be arrived at with regard to the possibility of stomatal infection, but obviously the most frequent method of infection is through wounds.

On the trees the spots are only found on ripe fruit, and two or three farmers have reported it as occurring only on fallen fruit or developing in the store room. The organism is very probably a soil bacillus which first invaded fruit lying on the ground and has now taken on a parasitic habit. It was found quite possible to infect green lemons, although the organism attacked them less readily than the ripe fruit.

The organism loses its virulence rather rapidly on artificial media; this was clearly shown by inoculating two similar sets of lemons, one with a freshly isolated culture, and the other with a culture isolated about a month previously and transferred fifteen times. On the first, signs of infection were visible after 48 hours and distinct spots in four days; on the second, the discoloration was much delayed; it was not visible till the fourth or fifth day and the infected areas comparatively small and poorly developed.

The identity of the organism in fruit and branch was also tested. A culture of this bacillus isolated from a leaf base infection was used to inoculate a number of sound lemons with positive results.

A strain isolated from lemons sent from the Constantia Wine Farm produced a few positive infections on some young trees growing in tins in the Laboratory grounds. From these the organism was again isolated and employed with positive results to infect a number of lemons.

Pathological Histology.

The organism was most easily studied in the rind of affected fruit; satisfactory preparations were obtained by fixing in hot acetic alcohol and staining with Ziehl's carbol fuchsin and light green.

This is essentially a disease of the parenchyma, the lignified tissues of the stem are not invaded, and the organism is not found in the small fibro-vascular bundles in the rind. The bacillus does not cause hyperplasia.

As has been stated, in the majority of cases infection takes place through a wound; the very slightest abrasion with a fine needle being sufficient. No definite evidence has yet been obtained by inoculation, that stomatal infections are possible, but in a number of sections, particularly those through incipient spots on naartje rind, the location of the bacilli strongly suggested that they had effected an entrance through the stoma. (Plate X, *a*.) In such cases the stomatal cavity was occupied by a mass of bacteria and the surrounding intercellular spaces had also been invaded. The rods multiply very rapidly in the intercellular spaces, until the latter become much distended and bulge into the cell cavity. Considerable tension is thus set up and eventually the wall gives way and the bacilli force an entrance into the cell.

The cell cavity soon becomes entirely filled, but for a considerable time the remaining walls are intact so that were it not for the absence of any intercellular spaces it would appear that the organism was intracellular. The cell contents become disorganised and disappear with the exception of the protoplasmic lining of the cell wall which contracts slightly away from the wall and stains deeply with fuchsin. The cells at this stage consist of a central mass of bacteria surrounded by a deeply stained sack of disorganised cell contents and then by the partially destroyed cell wall. The staining reaction of the latter is not altered. Finally the intervening walls give way and the tissues become completely disorganised. (Plate X, *c*.)

The oil gland is often completely filled with a vast number of rods; it was afterwards found that the organism grows extremely well in the presence of orange oil and lemon oil. The cells surrounding the oil glands are also very frequently attacked.

Morphology.

The organism is a rather slender rod with rounded ends; the living rods diffusing from discoloured cells in the rind of a lemon measured ·8 to 3·2 μ by ·5 to ·7 μ; in a smear from the same source stained with dilute fuchsin they measured 1·4 μ by ·45 to ·7 μ, the majority in each case were about 1·5 × ·5 to ·6 μ. Similar measurements were recorded for rods from diseased tissues of spots on oranges and naartjes. The bacilli in the viscid mass oozing from affected fruit are slightly stouter on the whole than those in the tissues, most of them are ·6 to ·65 μ in thickness, in length there is not much variation.

Measurements of rods from a 24 hour culture on nutrient agar and in nutrient broth coincided with those of rods taken direct from the tissues of the host plant, but on gelatine they are considerably shorter, the majority measuring ·8 to 1 μ by ·5 to ·6 μ. In Uschinsky's solution short forms also predominate.

In old agar streaks the rods vary very much in length, some are almost spherical, others attain a length of 5 μ before dividing; some stain intensely, others faintly or unevenly.

No long filaments have been observed in the pellicle on sugar broth, the longest unsegmented forms noticed in these media were about 8 μ in length.

The sediment in three months old broth cultures consists for the most part of rather short rods, some of which stain intensely with gentian violet, but the majority stain faintly or unevenly. In old cultures on steamed potato there are also a large number of individuals which only stain faintly, and these are considerably swollen so as to be ellipsoid or almost spherical in form.

Fission. The multiplication of the organism by fission was studied on the agar hanging block; a smear was made from a 24 hour old broth culture which had been incubated at a temperature of 35° C., the preparation was illuminated by a Nernst lamp, and the lenses used were a Zeiss $\frac{1}{12}''$ oil immersion objective and No. 12 compensating ocular. With this magnification the alteration of the rods in size and form could be easily followed, and drawings were made with the camera lucida at frequent intervals. The temperature of the room was approximately 26° C.

At 9.15 a.m. a single rod was selected for study (Plate XII, *a*). It at once elongated somewhat, became constricted and by 10 a.m. had divided into subequal rods. The upper of the two new individuals

thus formed had divided again by 10.45, the lower became constricted and divided soon after. Subsequently each rod took approximately 20 minutes after separation to attain to its maximum size, and then immediately became constricted and fission was complete in another 20 minutes. Each individual became slightly curved or bent when it became constricted previous to fission, and when division was completed the resulting rods were not exactly equal. The newly formed individuals at once became separated by an appreciable gap, their close contact being prevented by the capsule which could be detected by a careful adjustment of the illumination.

At 1 p.m. the young colony consisted of 16 rods; by 1.30 the number had increased to 22 and it was no longer possible to make accurate drawings as many of the rods had oriented themselves with their long axes parallel to the line of vision and they were overlapping in various directions.

No further observations were made until 9.15 on the following morning; at that time the surface of the block was covered with highly refractive moruloid masses of bacteria; the capsuled bacteria in each mass were surrounded by a common envelope with a high refractive index, and outside of this were a large number of rods lying free (Plate XII, *b*). When first examined the free rods round the masses near the edge of the block which were probably better supplied with oxygen than the rest were in active motion, and large numbers of them had made their way from the agar to the surface of the cover glass beyond which was covered with a thin film of moisture. After about 15 minutes' exposure to brilliant illumination from the Nernst lamp all these rods came to rest.

Grouping. The rods are most commonly single or in pairs; loose chains are found in the pellicle and also in sediment in some sugar broths, particularly on nutrient broth with 2 per cent. laevulose, but these do not as a rule consist of more than 20 or 30 elements (Plate XIII, *a*) and they lie amongst large numbers of single rods. There are no chains in the ring above the liquid.

On solid media the organism almost invariably forms itself to a greater or less extent into the capsuled, moruloid masses described above, they are also found in the pellicle on ordinary nutrient broth. If such masses be mounted in water they do not resolve themselves into chains but isolated rods disentangle themselves and swim away in a state of high activity.

Motility. The organism is often sluggishly motile when it is taken

direct from the tissues of the host, and if a small portion of the viscid mass oozing from affected areas on the fruit be mounted in water the rods diffuse out and immediately become very active.

It is extremely active in young cultures; an examination under the low power of the microscope of 18 to 24 hour old colonies on nutrient agar shows the outer part of each colony as a swirling mass of actively motile rods. In liquid media the bacillus retains its activity for a considerable length of time; chains from the pellicle of a 16 days old broth culture separated and immediately became very active on being transferred to water.

The motility of the organism can be very well studied in preparations made from a young agar culture and observed with the dark ground illumination. The rods move forward with a steady sinuous motion which is frequently interrupted by rapid oscillating movements.

The flagella stain quite readily; in the living state they may be stained by Straus' method(1). The position of the flagella can be well made out, they are obviously peritrichous but are in too rapid motion for any accurate estimate of their number. With Ellis' modification of Loeffler's method very satisfactory preparations were obtained. There are 5 to 10 long peritrichous flagella: these are several times the length of the rod, measuring roughly 8 to 12 μ. They are not distributed evenly over the surface but frequently consist of one or two groups of 3 to 5 which are crowded up towards the poles (Plate XII, *c*).

Capsules. The viscidity of the substance oozing from diseased tissues and the leathery or mucilaginous texture of many cultures on solid media at once suggest the presence of a capsule.

A capsule may almost always be detected and may be stained either by one of the special capsule stains, by a dilute solution of fuchsin or with carbol gentian violet. The viscid drops oozing from the fruit consist of capsuled rods embedded in a tenuous slime which appears granular when stained by MacConkey's method (Plate XIII, *d*).

In an impression preparation of a young agar colony all the rods were definitely capsuled (Plate XIII, *c*) and in the older parts of the colony become crowded together into small tough masses which are firmly embedded in the medium. In certain cases streak cultures on agar take on a roughened or shagreen-like appearance, and the streak becomes leathery and can be torn in a strip from the surface of the medium. Old streaks of raised, shining, more homogeneous appearance are usually mucilaginous; the difference in texture appears to have some relation to the amount of water present in the medium.

No capsules have been observed around rods within the tissues of the host.

The capsule around each rod in an impression preparation stained with fuchsin measures roughly 1·5 μ in diameter and it exceeds the rod in length by about ·5 μ at each end. A few rods may be seen without capsules and empty capsules are quite common, but it was not clear whether the rods escape from the capsules or whether they had been torn away in making the preparation.

Involution forms were observed in broth cultures containing high percentages of sodium chloride. They are very common in 48 hour old cultures in broth containing 9·5 per cent. to 10·5 per cent. NaCl. The rods comparatively are very large, some grow out into filaments as much as 100 μ long, and they are irregularly swollen and contorted in every direction (Plate XIII, *e*). Quite a number are club-shaped. They stain intensely with aniline gentian violet and when transferred to ordinary nutrient broth rapidly divide and produce rods of normal form.

Aberrant forms are also found in old potato cultures. A smear made from a 12 days old potato culture consists of very minute intensely staining capsuled forms, some of which are almost spherical and slightly swollen rods of normal length which stain very feebly or irregularly. The latter were only faintly stained in half-an-hour by carbol gentian violet (Plate XIII, *b*).

Staining reactions. The bacillus stains well by all the ordinary methods, but varies considerably in its reaction to the various dyes. In testing the action of the different stains smears were made from a 24 hour old streak on nutrient agar. Dilute aqueous solutions were allowed to act for two minutes; in this time the thionine and methylene blue hardly stained the rods at all, fuchsin gave a distinct though rather faint coloration and the rods stained with gentian violet were very intensely and quite sharply stained. Carbol gentian violet also gave a very strong intense stain in half a minute and brought out the capsules which were not stained by the aqueous solution. Ziehl's carbol fuchsin gave a similar result; carbol methylene blue and carbol thionine also stained very faintly in half a minute, but Loeffler's blue gave much better results in the same time though not nearly so intense a stain as the gentian violet and fuchsin.

The organism is Gram-positive, and stains blue by the Ziehl Neelson method, *i.e.*, it is not acid fast.

Spores. No spores have been observed.

Cultural Characters.

Nutrient agar colonies. The organism grows very rapidly on nutrient agar and when first isolated from the tissues the speed with which the colonies appear varies considerably; after preliminary cultivation however, more even results are obtained. The following is a description of the development of typical colonies at 25° C.

After 24 hours in thinly sown plates the colonies are 1—4 mm. in diameter, they average 2 mm., and are round-irregular in shape; by transmitted light translucent with a small opaque centre, pearly white to bluish, yellowish by reflected light. Under the microscope the colony is granular with a grumose centre, the granular margin being composed of a swirling mass of actively motile rods. Submerged colonies are minute opaque, mostly bi-convex.

After two days the average diameter of the colonies has increased to 4 mm., and some measure as much as 7 mm., they are coppery by transmitted light with a bluish margin, straw yellow by reflected light. There is a small opaque centre surrounded by a rather dense sub-opaque ring and then by a translucent margin. Under the low power of the microscope the central portion is grumose, the outer finely granular.

Submerged colonies have increased somewhat in size and now have the appearance of two or more lenticular bodies placed at different angles, or of a single lenticular body with sub-spherical outgrowths.

After three days the colonies average 5—6 mm. in diameter, the largest are 10 mm., they are round-irregular, umbonate; Naples yellow to buff yellow by reflected light, coppery throughout by transmitted light; under the microscope they are much more dense than before except at the edge, and the whole of the central part is grumose; there is no longer any motion visible with a low magnification. Submerged colonies have increased in size up to $\frac{1}{2}$ mm.

After four days the colour has become mustard yellow and long feathery crystals have made their appearance in the medium.

There is usually no further change after the fifth day; the colonies then measure up to 15 mm. in diameter, the margin is entire or undulate, and there are frequently two or three concentric rings. The surface of the agar has become distinctly whitened especially in the immediate vicinity of the colonies.

The growth in this case does not become tough or leathery, it is always creamy in consistency.

The size of the colonies is very much affected by crowding. (Plate XIII, *b*, *c*, *d*.)

Nutrient agar streak. A well developed agar streak appears in 24 hours at 25° C.: it is smooth, shining, about 3 mm. wide and grumose in the centre. In three days this has become 3—5 mm. broad, primuline yellow, the central part being pulvinate, and the margin entire or slightly undulate.

After 12 days the surface of the agar has become very decidedly whitened except for the parts covered by the bacterial growth; long feathery crystals point downwards into the medium from the under surface of the streak. The condensation water is very heavily clouded. A second kind of crystal is formed on the surface of the agar under the condensation water, very similar to those illustrated by Smith as occurring in cultures of the olive tubercle organism (7).

The texture of the streaks in three weeks old culture varies very much with the amount of water present in the medium and in the atmosphere. Some remain creamy, but contain small tough moruloid masses; others become very tough at an early stage, large strips may be pulled away on the platinum needle and when this is done the growth stretches somewhat and has the appearance of a number of spherical colonies in a tough homogeneous matrix. Most commonly the growth is raised and almost homogeneous to the naked eye. It is then very mucilaginous, and in colour and texture is not unlike plastic sulphur.

If the surface of the sloped agar be bathed in lemon or orange oil a very luxuriant growth is obtained which does not become tough or mucilaginous.

Nutrient agar stab, the best growth is at the surface.

Glucose formate agar. Streaks were made on this medium as a control for those kept under anaerobic conditions; the growth is very similar to that on ordinary nutrient agar, but slightly more luxuriant.

Sulphindigotate agar. This medium was used for a similar purpose; a very luxuriant growth was obtained, on the upper drier portion of the slope a number of small discrete colonies were produced which at first absorbed the colouring matter from the medium and became blue. The lower part of the slope was almost covered by a wet shining, yellowish growth which was grumose in the centre. The colour gradually disappeared from the medium.

Lemon and *Orange glucose agar.* Colonies on these media are similar to those on nutrient agar but considerably smaller, only 2·3 mm. in diameter after three days; they are very markedly umbonate in

the centre, and concentric rings are visible by transmitted light; under the low power of the microscope they are grumose in the centre and coarsely granular near the edge; in colour they are dirty yellowish white. After five days the diameter has increased to 5 mm., and the colonies have a number of distinct concentric zones, by transmitted light the two central zones are yellow and the remainder bluish white.

Lemon and orange glucose agar streaks[1] are dirty yellowish white, glistening, very slightly raised, 3—10 mm. broad, and the condensation water is rather heavily clouded. On the lemon glucose agar the streak had a shagreen surface, on orange glucose agar the surface was smooth but moruloid in the centre.

Orange laevulose agar. Streaks on this agar are similar in character to those on orange glucose agar but are considerably more luxuriant.

Beerwort agar. The organism grows very well on this medium, it forms a shining streak with a convex central portion and a flat margin with a slightly undulate edge; the breadth of the central portion is 4—5 mm., of the margin 1 mm. There is a copious yellow growth in the condensation water.

As it dried up the surface of the raised central part becomes corrugated and it is decidedly viscous.

Bean agar is a fairly good medium, streaks are smooth, yellowish, somewhat umbilicate, and about 3 mm. diameter.

Cabbage agar. A very characteristic growth forms on streaks of cabbage agar. It is not very abundant and consists of a number of spherical, opaque, yellowish colonies standing up above the surface of the medium, and disposed irregularly in a flat translucent streak 1—4 mm. wide. The surface of the agar becomes conspicuously whitened in the vicinity of the streak.

Loeffler's blood serum is not liquefied, the organism forms a shining yellow growth along the needle track.

Starch jelly. No growth.

Nutrient gelatine colonies are just visible to the naked eye after 24 hours at 20° C. as minute glistening points. In two days they attain a diameter of 1—3 mm., and are round, glistening, slightly convex, coppery by transmitted light, creamy by reflected light. Under the microscope they are rather coarsely granular and inclined to become

[1] *Orange or Lemon glucose agar* was made as follows: 50 grammes of fresh rind was boiled for 30 minutes with 500 c.c. distilled water, filtered, then 10 grs. dextrose added and solidified in the usual way with 1·5 agar powder.

In *Orange laevulose agar*, laevulose was substituted for *glucose*.

grumose in the centre. Submerged colonies are minute, white and subspherical.

Seven days old colonies are up to 4 mm. diameter, apricot yellow, they are beginning to sink in the centre owing to the softening of the gelatine, the growth is slightly viscous. In nine days the gelatine in the immediate vicinity of the colonies is liquefied, and in 14 days liquefaction is complete. The colonies are still almost intact and floating in the liquefied medium.

Nutrient gelatine shake cultures develop very numerous minute colonies, the majority of which are within 1 cm. of the surface of the medium, which is slowly liquefied.

Nutrient gelatine stab. The organism grows comparatively slowly at 20° C., the growth along the needle track being barely visible at the end of 24 hours. After seven days there is a fairly thick line of growth all along the needle track, the surface colony is slightly sunken in a small crater of liquefied gelatine. In 14 days liquefaction is napiform, the surface growth sinks to the bottom of the liquefied gelatine, continually re-forming and sinking, thus forming a considerable amount of sediment. The liquefied portion is clouded, and there are very numerous minute opaque yellowish flocculi in suspension throughout it. (Plate XI, *a*.)

After 30 days the liquefaction is broadly saccate and extending to the bottom of the tube, and in 6—7 weeks the gelatine is completely liquefied.

Nutrient gelatine streak. In three days there is a shining yellowish streak about 2 mm. wide and slightly raised in the centre. Liquefaction begins on the fourth day; a small pocket of gelatine liquefying near the bottom of the streak. The following day there is a groove along the surface of the slope, from which the liquefied gelatine has run carrying the yellowish growth to the bottom of the tube. A fresh streak forms in this groove, in its turn to be carried to the bottom of the tube, and this process is repeated several times. The yellow growth does not diffuse readily into the liquefied medium.

Potato. On potato the bacillus grows well, a thin spreading yellowish growth almost covers the sloping face of the cylinder in 24 hours at 25° C.; after three days the growth is primuline yellow to mustard yellow, glistening, spreading, covering all the lower part of the cylinder except where it is in contact with the tube and the greater part of the upper drier portions. No growth is visible in the liquid in the bulb. The potato is distinctly browned, the discoloration being most marked in the tubes containing glycerinated potato.

Carrot. The water in the bulb of the Roux' tube is distinctly clouded, on the carrot there is a plentiful, colourless wet-looking, shining growth spreading over the medium.

Turnip. Growth is much less abundant than on carrot, it is not quite colourless but has a yellowish tinge.

Beet. Here the growth is very similar to that on carrot but is distinctly yellow, and there is a yellowish ring at the surface of the liquid in the bulb of the Roux tube.

Orange and lemon rind steamed show after three days glistening, dirty yellowish masses of slightly viscid bacilli raised above the surface of the rind.

Nutrient bouillon (+ 15 Fuller). Nutrient broth clouds in six hours at temperatures from 25° to 35° C., and in 24 hours there is a distinct suggestion of pellicle formation. The pellicle is thin and falls to the bottom of the tube if the latter is shaken slightly. Three months old cultures are almost clear; there is a small amount of dried up growth on the tube where the surface of the medium originally was and a considerable amount of deposit. The rods in the deposit are living and multiply readily when transferred to fresh tubes of broth.

Pellicle formation takes place most readily on broth with an acid reaction, on alkaline broth little or no pellicle is formed. After standing for some days flasks of acid broth showed a thin pellicle in which were suspended numerous more opaque portions which are shining, yellowish and rather greasy looking. In some lights the pellicle is distinctly iridescent.

The ring above the liquid does not become tough or mucilaginous, and the pellicle is flocculent.

Sugar bouillons. The growth in dextrose broth is not particularly good; a heavier clouding was obtained in broth containing maltose, lactose and galactose, and the best growth of all in 2 per cent. laevulose broth.

Litmus milk. In milk the bacillus grows comparatively slowly; no change could be observed for seven days, after that period the milk became slightly more acid in reaction and the litmus was then gradually reduced. After 10 days at 35° C. the casein coagulated and there was a gradual extrusion of whey. The whey was not abundant, almost clear, and yellowish in colour, a sediment of yellow bacteria gradually accumulated on the surface of the coagulum; the latter did not become peptonised.

Milk serum, filtered through a porcelain candle, proved to be

a favourable medium. The liquid became turbid and a decided whitish pellicle formed on the surface.

Dunham's solution became only very slightly clouded; no pellicle and very little sediment.

Cohn's solution. No growth.

Uschinsky's solution at first is only slightly clouded but a thin tough pellicle forms; this increases in size, remaining adherent to the sides of the tube and becoming saccate and dipping below the surface of the solution in the centre of the tube. This pellicle finally sinks to the bottom and the liquid then becomes densely clouded and baryta yellow to buff yellow in shade. There is a decided ring above the liquid which becomes ochraceous tawny to cinnamon brown.

Cabbage broth. There is not much clouding and only a small amount of sediment. The pellicle however is quite conspicuous, it consists of vast numbers of minute, opaque, yellowish bodies and presents a frosted appearance.

Lemon juice filtered through a porcelain candle and undiluted proved to be too acid a medium for growth.

Nitrate broth is heavily clouded but no pellicle forms owing to the vigorous evolution of gas. The broth is covered with froth to the depth of 1 cm., for some days.

Physical and Biochemical Features.

Enzyme production.

Proteolytic enzymes. Milk serum cultures tested at the end of five days gave only negative results; after 10 days however, although the culture gave negative reactions for proteose and peptone there was a faint but unmistakable reaction for tyrosin which was absent in tests made from the control flask.

Diastase. Potato cylinders on which the organism had been grown gave a distinct red brown reaction with iodine indicating the presence of amylodextrin. The same reaction was observed in peptone water containing potato starch, but in neither case was there any reaction for sugar with Fehling's solution.

Nutrient bouillon cultures to which were added 2 per cent. thymo and equal quantities of starch paste did not precipitate Fehling's solution after six to eight hours.

Invertase was also absent from broth cultures.

Acid production. In nutrient broth containing 2 per cent. dextrose, laevulose, saccharose, lactose and glycerine and in ordinary broth without the addition of any carbohydrate there is no acid production, but the medium in 10 days becomes slightly more alkaline, but only by 10 degrees or less of Fuller's scale.

In peptone water tinted with litmus, however, the organism produced acid with several carbohydrates. The following table shows the behaviour of the organism in tubes containing Dunham's solution and various carbohydrates and other substances.

Dextrose	Slightly acid, litmus somewhat reduced
Laevulose	Acid reaction marked
Galactose	Slightly acid, litmus considerably reduced
Maltose	Distinctly acid
Lactose	No change in reaction
Saccharose	Slightly acid, litmus somewhat reduced
Dextrin	No change in reaction, litmus completely reduced
Starch	No change in reaction
Glycerine	No change in reaction
Mannite	Distinctly acid
Sodium formate	No change in reaction
Sodium citrate	No change in reaction

The bacillus therefore produces acid in the presence of laevulose, dextrose, galactose, maltose, saccharose and mannite, most marked with laevulose.

Alcohol production. The first distillate from a culture in 2 per cent. laevulose broth was tested for alcohol, etc. The distillate was divided into four portions, to the first was added Lugol's iodine, then a little NaOH solution; on heating the mixture there was a distinct smell of iodoform. The second portion tested with Schiff's reagent gave a negative reaction for aldehyde. To 10 c.c. of a third portion was added 2·5 c.c. 25 per cent. sulphuric acid and a crystal of potassium bichromate. This was distilled, and in the process the bichromate was reduced to a green colour, and the distillate smelled strongly of acetaldehyde and reacted with Schiff's reagent.

A similar test with distillate from control flask gave only negative results. It is evident therefore that an appreciable amount of alcohol is produced in sugar broth cultures.

Ammonia production. The distillate from cultures in nutrient broth gave a positive reaction for ammonia with Nessler's solution.

Indol is produced both in Dunham's solution and in nutrient broth incubated for 10 days at 25° C., but all tests for *Phenol* were negative.

Pigment production has been described in various media in connection with the cultural characters of the organism. The pigment is insoluble in water (hot and cold), alcohol, ether, chloroform, carbon bisulphide and dilute acids and alkalis.

Reducing agent formation.

Colour reduction. The reducing power of the bacillus is very slight; it was tested in nutrient broth tinted with various coloured solutions. Rosolic acid and indigo carmine are not reduced. Methylene blue becomes peacock green in two days and in five days is completely reduced. Neutral red which was at first flame scarlet in four days was reduced to orange buff but was completely reduced only at the end of 15 days.

Litmus showed no sign of reduction until after 15—21 days when there was a small amount of colourless liquid at the bottom of the tube, after a few days the colour again became even.

Reduction of nitrates. In nitrate broth the nitrates are reduced with evolution of gas, the evolution being most vigorous at 25° C. from the second to the fourth day, on the fifth day it ceased. The tubes were tested at the end of the tenth day and gave no reaction for nitrate or ammonia.

In nitrate peptone water no gas is evolved and after 10 days the solution gives positive reactions for both nitrates and ammonia.

In control tubes both tests were negative.

Gas production. No gas is evolved in fermentation tubes containing bouillon or peptone water and any of the substances mentioned in connection with acid production. The organism grows in the closed end of the fermentation tube but frequently the clouding is only slight. In iron peptone and lead peptone solution there was a distinct blackening of the precipitate as compared with the control tubes; a small amount of sulphuretted hydrogen is therefore present.

Atmosphere. The organism is a facultative anaerobe, as suggested by its ability to grow in the closed end of the fermentation tubes.

Four sets of cultivations were prepared, one of which was sealed in Buchner's tubes containing an alkaline solution of pyrogallic acid, and the other three were each placed in a Bulloch's apparatus.

From the first the air was exhausted as completely as possible so that the organism was growing under reduced pressure; from the second the oxygen was absorbed by pyrogallic acid, and the air in the third was displaced by CO_2.

The bacillus grew almost as luxuriantly under reduced pressure as in the control tubes. In the absence of oxygen, *i.e.*, practically in an atmosphere of nitrogen, and in CO_2 growth was comparatively very slow and restrained.

Temperature. The bacillus grows through a wide range of temperature, and very rapid multiplication takes place at all temperatures from 25° C. to 38° C. The optimum temperature is 35° C. At 15—18° C. the organism grows very slowly, and at 0° C. no clouding can be observed in tubes of nutrient broth. The organism is not killed by long exposures to this temperature, but rapidly clouded tubes transferred to a warmer temperature after 10 days. The upper limit for growth is 43° C., broth is not clouded at 45° C. and the bacillus is destroyed by several days exposure to this temperature.

The thermal death point (in standard 10 c.c. tubes 10 minutes exposure) is 62° C.; dry on cover slips it is 110° C.

Reaction of medium. The organism is not very sensitive to the reaction of the medium and grows almost equally well in broth with reactions of + 15 to + 25, the optimum however is about + 20 of Fuller's scale. It grows through a wide range and can grow in a medium of fairly high reaction if the acid used is malic, citric or tartaric.

Substance tested	Maximum of growth	Amount to inhibit growth
Acetic acid	+25	+ 30
Oxalic acid	+25	+ 30
Citric acid	+95	+100
Tartaric acid	+95	+100
Malic acid	+95	+100
Sodium hydrate	−40	− 45

Toleration of NaCl. The percentage of salt which the organism can tolerate is surprisingly high; some slight growth took place in tubes containing 10·5 per cent. NaCl and those with 10 per cent. were decidedly clouded. Involution forms are however produced in broth containing high percentages.

Desiccation. A young agar culture was suspended in water and smeared on cover slips. As soon as the smear was dry, these were transferred to a desiccator; they were removed from time to time and dropped into tubes of nutrient broth. The organism was still alive after 80 days, more prolonged tests have not yet been carried out.

Sunlight. The test was carried out in very bright sunlight soon after midday in the month of October. Thinly sown plates were

employed, one half being covered with black paper. To prevent the effects of a rise in temperature, each plate was covered with a small glass dish filled to a depth of 2 cm. with a 2 per cent. solution of potash alum. The organism was killed by an exposure of 60 minutes and the number considerably reduced in 45 minutes.

Chloroform. The bacillus will not grow in nutrient bouillon over chloroform.

Germicides. The following table will serve to indicate the behaviour of the organism toward the more common germicides:

Substance	Maximum for growth	Amount to inhibit growth	Amount to kill organism in 30 minutes
Copper sulphate	1 : 10000	1 : 5000	1 : 1500
Carbolic acid	1 : 1000	1 : 500	1 : 100
Mercuric chloride	1 : 10000	1 : 5000	1 : 4000
Formalin	1 : 1000	1 : 750	1 : 50

It will be observed from the above schedule that the organism is very sensitive to copper sulphate, it is to be hoped therefore that good results may be obtained by spraying the infected trees with Bordeaux mixture.

Pathogenicity to Animals.

Through the courtesy of Sir Arnold Theiler, the pathogenicity of the organism to animals was tested at the Veterinary Research Laboratories at Onderstepoort. I am indebted to Mr E. M. Robinson for the following notes on experiments which he has carried out in this connection.

To test the pathogenicity of the Bacillus of Lemon disease, two rabbits were inoculated each with half an agar slope of a 24 hours' growth of the organism. One rabbit which will be called (A) was inoculated on 25th September, 1915, and the inoculation was given intraperitoneally. This rabbit developed symptoms of extreme illness and on the two days following the inoculation would not eat, refused to move and occasionally made grinding movements with its teeth. On the third day after the inoculation the rabbit appeared much less dull and at the end of a week appeared quite well again. On the 7th October, 1915, the rabbit received a further inoculation of half an agar slope (24 hours' growth) subcutaneously, but showed no further symptoms of illness, and is still healthy to date (14th November, 1915).

Another rabbit (B) was inoculated with half an agar slope of the organism (24 hours' growth) subcutaneously on the 25th September, 1915.

It developed no symptoms of illness and on 7th October, 1915, received a further similar subcutaneous inoculation, the same dose being used. On 11th October the rabbit showed symptoms of severe illness with thick mucopurulent discharge from the eyes and extreme dullness. On 12th October, 1915, it was found dead, and the post mortem examination showed nothing but a slight hyperaemia of the lungs. Cultures from the heart's blood remained sterile. This rabbit's death was probably not due to the bacillus of Lemon disease as the inoculation of the first rabbit (A) was a much more severe test of pathogenicity.

Intravenous inoculations were not attempted as it has been found that most non-pathogenic organisms will produce death from toxaemia except in very small doses.

Rabbit (A) was bled on 12th November, 1915, and its serum tested for agglutinins against the organism. Agglutination was obtained in a dilution of 1—800 distinct, and partial in a dilution of 1—2000. The serum of a normal rabbit gave no agglutination in as low a dilution as 1—10. Fresh orange juice would not agglutinate the bacillus in any dilution.

The agglutination test should prove of use in the ultimate proof of organisms causing plant diseases and is quite easy to apply.

Comparative table of characters of three organisms causing diseases of Citrus.

	Bacillus citrimaculans	Bacterium citriputeale	Pseudomonas citri
Nature of disease produced	Dark sunken spots	Dark sunken spots	Canker
Organs affected	Fruit, stems, rarely leaves	Fruit	Fruit, stems and leaves
Form of organism	Rod with rounded ends	Rod	Rod with rounded ends
Dimensions	1—4 × ·45—·7μ	2—4 × ·51—1μ	1·5—2 × ·5—·75μ
Flagella	5—10 peritrichous	1 polar	1 polar
Capsule	Conspicuous	None	Not recorded
Colonies on nutrient agar	Sub-circular, yellow	Greyish white to pearl grey	Circular yellow
Gelatine stabs	Liquefaction napiform	Liquefaction stratiform	Liquefaction filiform
Milk	Becomes slightly acid, casein precipitated with gradual extrusion of whey	Becomes intensely alkaline and clear without separation of casein	Becomes alkaline, casein is precipitated, whey clear
Test for indol	Positive	Positive	Negative

Résumé of Salient Characters.

Bacillus citrimaculans, n.sp. Causes spot disease of citrus, attacking fruit and branch, rarely found on leaves; attacks the parenchyma, first occupying the intercellular spaces then breaking down the cell walls; in nature attacks lemons, oranges and naartjes, and in addition has been successfully inoculated into limes, shaddock, grape fruit, citron; seville oranges are resistant.

A slender rod with rounded ends, 1—4 × ·45—·7μ, average 1·5 × ·5 to ·6μ; motile by 5—10 peritrichous flagella; conspicuous capsule but no spores; involution forms in broth containing high percentages NaCl; stains readily by usual stains and exceptionally well with gentian violet and by Gram's method.

Forms showing sub-circular yellow colonies on nutrient agar with dense, grumose centre; liquefies gelatine, clouds nutrient broth, forming pellicle and sediment; does not liquefy blood serum; coagulates milk with precipitation of casein and extrusion of whey, the coagulum is not redissolved; no growth in Cohn's solution; characteristic growth in Uschinsky's solution.

No gas in fermentation tubes, acid with dextrose, laevulose, galactose, maltose, saccharose and mannite, but not with lactose, glycerine, dextrin, or starch. Nitrates reduced with evolution of gas; tolerates up to 10 per cent. NaCl; no growth in broth over chloroform, indol produced in media containing peptone.

Aerobic, facultative anaerobe; killed by 60 minutes exposure to sunlight; optimum reaction about 20 Fuller; T.D.P. 62° C., maximum for growth 43° C.

Group number 221.2321523.

Summary.

This paper gives an account of a citrus disease which is causing considerable trouble in the Western Province of the Cape. It causes dark sunken spots on fruit and shoot, and not only disfigures but provides an entrance for fungous parasites which completely destroy the fruit.

The causal organism is described at some length, and as it appears to be one hitherto undescribed has been named *Bacillus citrimaculans*.

The question of preventive measures is not discussed, but an

improvement in the sanitation of the orchard would certainly prove beneficial, and it is intended to carry out spraying experiments during the ensuing season.

Botanical Laboratories of the Union of
South Africa, Pretoria.

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EXPLANATION OF PLATES.

All drawings were made with the aid of the camera lucida.

PLATE

III. Lemons, natural infection from Simondium, C.P., (*a*) lemon with a single typical spot, (*b*) the fruit on the right has been wounded by numerous scratches.

IV. Lemons, natural infection, from Simondium, (*a*) an early stage of infection, the spots are still light coloured. (*b*) Numerous infections at and remote from stalk coalesced to form irregular bodies.

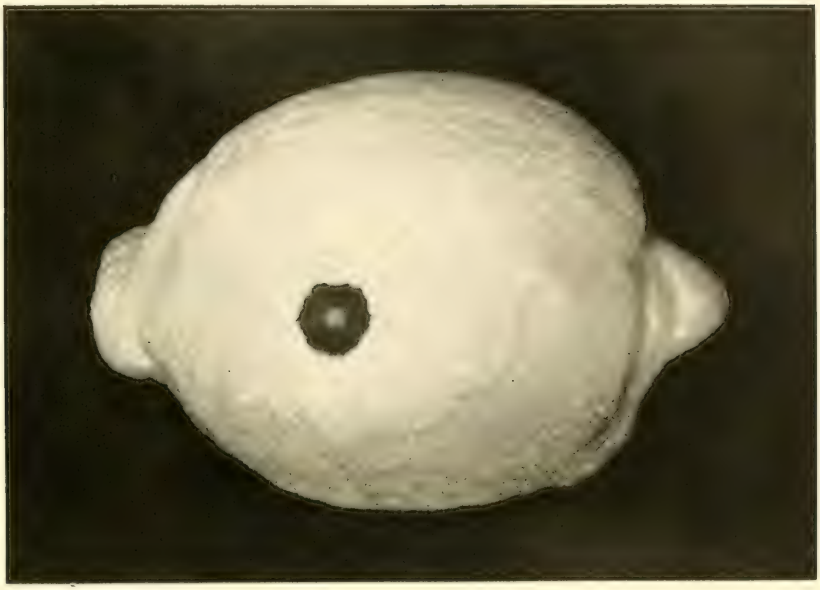
V. Lemons, artificial infection, pricked with sterile needle, then three of them atomised with suspension of a pure culture of *B. citrimaculans*; the fruit to the right in (*a*) is a control pricked with a sterile needle only.

VI. Lemons, artificial infection, ten days after inoculation, (*a*) inoculated by needle pricks, (*b*) with hypodermic syringe.

VII. Naartjes (*a*) artificially infected by needle pricks, (*b*) natural infection from Simondium.

VIII. (*a*) Navel oranges, natural infection from Simondium.
(*b*) "Parson Brown" seedling orange, artificial infection.

IX. (*a*) Lemon twigs showing infections round leaf bases, and petioles of fallen leaves.
(*b*) Small branch from orange showing the same characteristic discolorations.
(*c*) Leaves with bacterial spot, these are apparently rare and associated with tearing of tissues by thorns.



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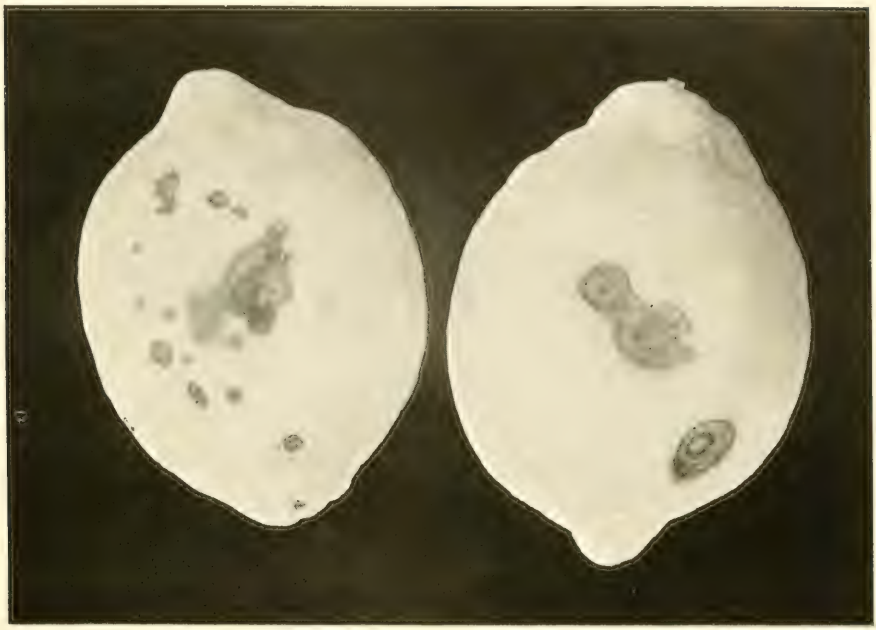

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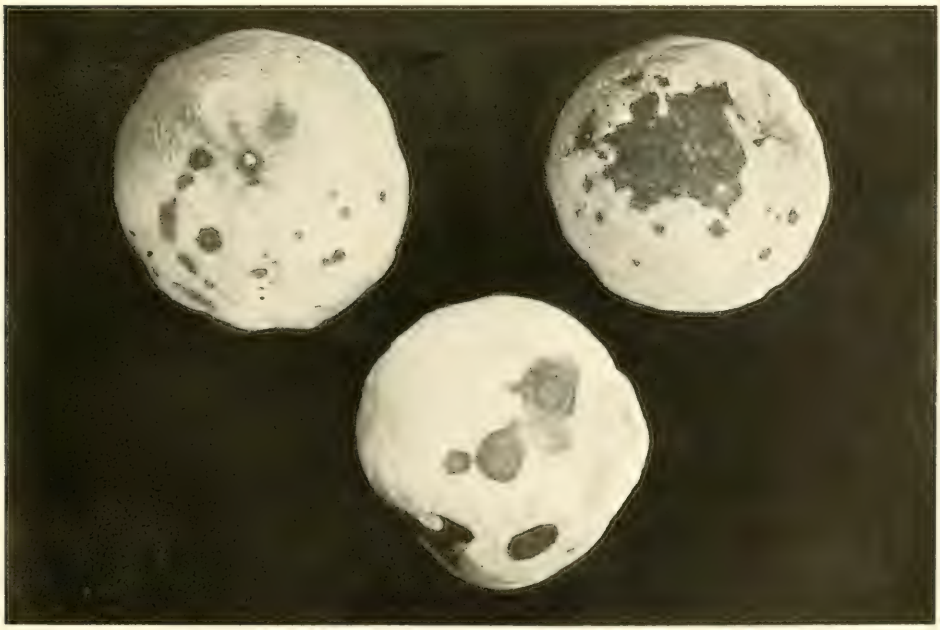

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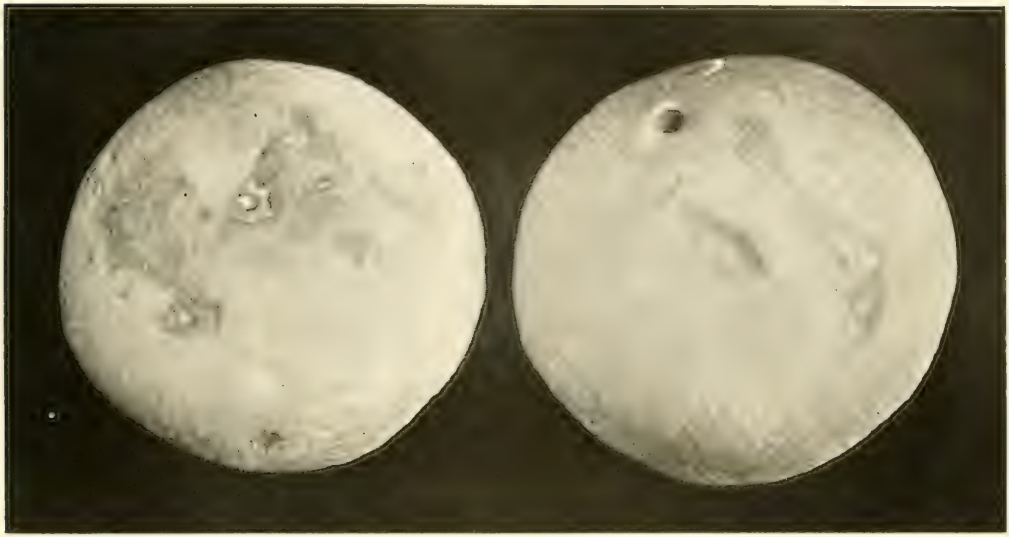

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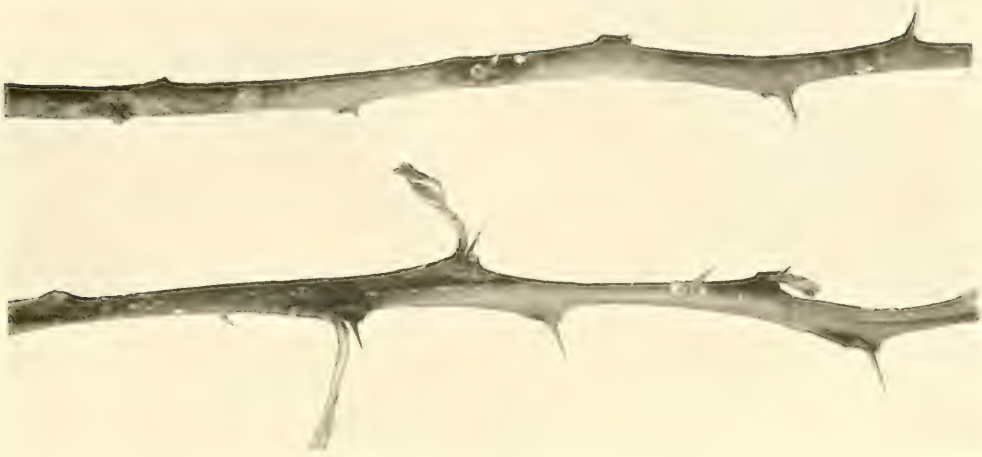

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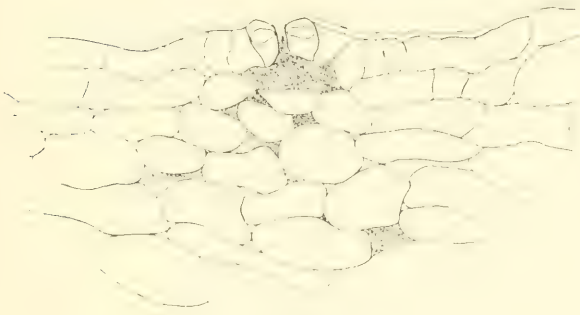

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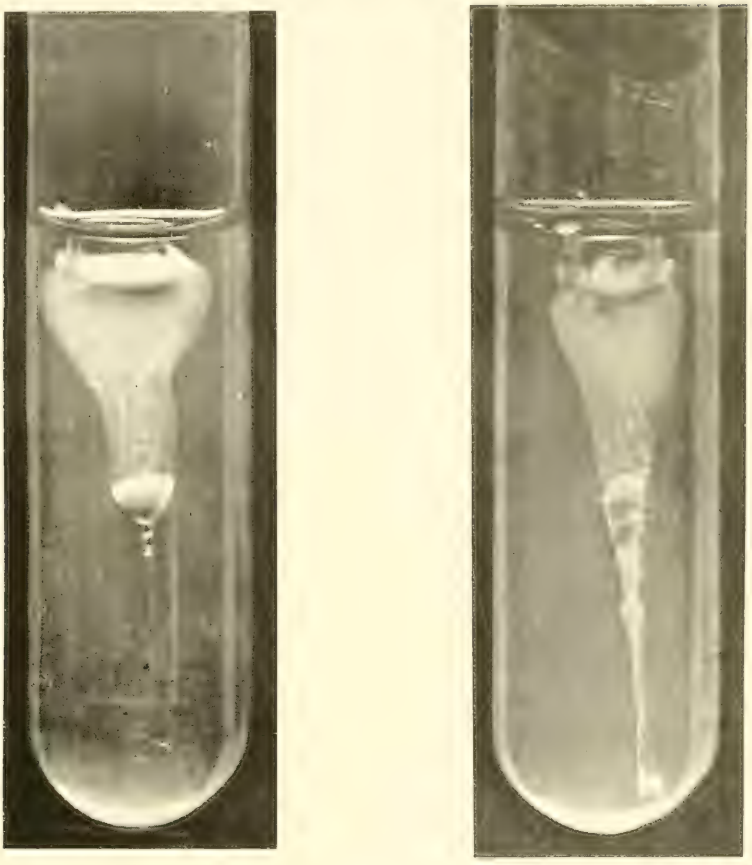

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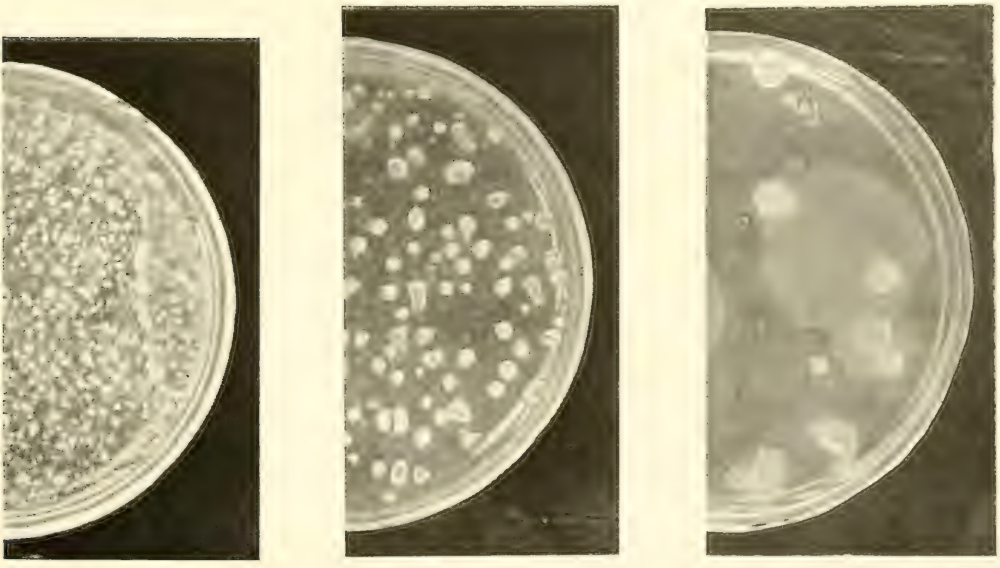

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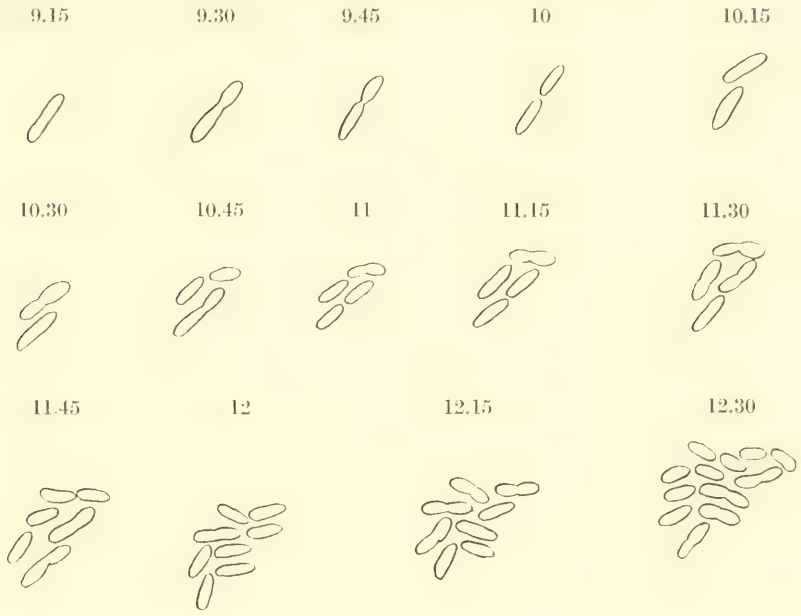

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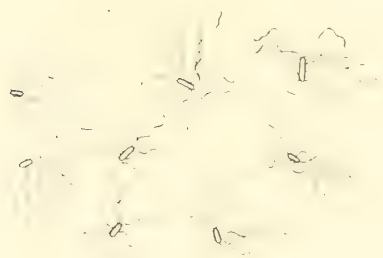

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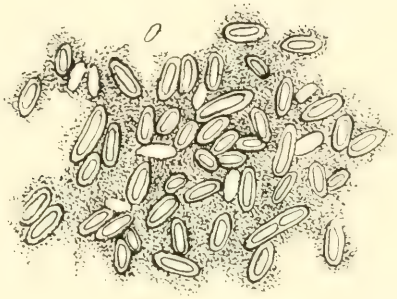

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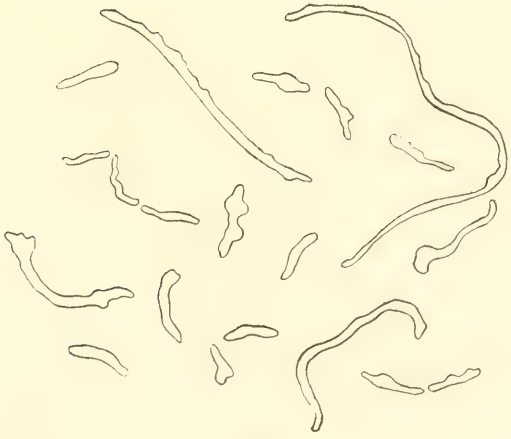

(*e*)

X. (*a*) Section through naartje rind at an early stage of infection showing the bacillus in sub-stomatal cavity and adjoining intercellular spaces. The size of the bacilli is not indicated. Zeiss $\frac{1}{12}$ mm., comp. ocular No. 6*.

(*b*) Section through lemon rind, bacillus has multiplied and distended intercellular spaces: same magnification as (*a*)*.

(*c*) Section through tissues of infected orange rind, showing cells completely disintegrated and occupied by masses of bacteria. Magnification as above*.

XI. (*a*) Stab cultures in nutrient gelatine after 11 days at 20° C., and 2 days at room temperature (about 25° C.). (*b*), (*c*) and (*d*) colonies on nutrient agar at 25° C. (*b*) and (*c*) 3 days old, (*d*) 5 days old. Shows the effect of crowding on the size of the colonies—somewhat reduced, diameter of petri dish, $3\frac{1}{2}$ inches.

XII. (*a*) Development of organism on agar hanging block: for full explanation see text (Zeiss $\frac{1}{12}$ oil immersion, comp. oc. No. 12. This magnification used for all drawings in Plates XII and XIII unless otherwise stated)*.

(*b*) 24 hour colony on hanging block (comp. oc. No. 6).

(*c*) Rods showing flagella stained by Ellis' modification of Loeffler's method. 18 hours at 25° C.

XIII. (*a*) Chains from pellicle on laevulose broth, 16 days at 25° C.

(*b*) Aberrant forms on potato cylinders, 12 days at 35° C.

(*c*) Capsuled rods from edge of impression preparation of colonies on nutrient agar, 18 hours at 25° C., stained with fuchsin.

(*d*) Rods in slime oozing from fruit, stained by MacConkey's method.

(*e*) Involution forms from broth containing 7·5 per cent. NaCl, 48 hours at 25° C., stained with aniline gentian violet.

* Exigencies of space have necessitated the reduction of the following figures:

Pl. X (*a*) by $\frac{1}{2}$: (*b*) by $\frac{1}{2}$: (*c*) by $\frac{1}{3}$.
Pl. XII (*c*) by $\frac{1}{2}$.
Pl. XIII (*a*) by $\frac{1}{2}$: (*c*) by $\frac{1}{4}$. [Ed.]

REPORT ON A TRIAL OF TARRED FELT "DISCS" FOR PROTECTING CABBAGES AND CAULIFLOWERS FROM ATTACKS OF THE CABBAGE-ROOT FLY.

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(With Plate XIV.)

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Introduction.

The present investigation has been carried out at the suggestion of Dr A. D. Imms. He has displayed a keen interest in the progress of the experiments and I have had the benefit of discussing with him any points that arose from time to time.

The expenses entailed during these experiments have been met from the annual grant of the Dept. of Agricultural Entomology, Manchester University.

It is a fact only too well known to most growers of cabbages, cauliflowers and related vegetables that large proportions of their crops are frequently lost owing to attacks of the Cabbage-root Maggot, the larva of *Chortophila brassicae*. A very large number of remedies have been devised with the object of either destroying the maggots

on infected roots, or preventing the deposition of eggs near the host plants. With one or two exceptions, however, these remedies are of little practical value since they require, as a rule, frequent application to the plants in order to obtain any satisfactory measure of success. For this reason, and also on the score of expense, most growers are averse to their use. Gibson and Treherne (6), in their recently published bulletin on the Cabbage-root Maggot, give a list of forty-eight insecticidal mixtures or protective measures which they have experimented with for several years. As a result of these experiments they make the following statement: "It can be truly claimed that the only protection to be relied upon for cabbages and cauliflowers and one which is in every way practical, is in the use of tarred felt paper discs."

The idea of placing paper collars round the stems of cabbage plants, and thus preventing egg deposition in the soil near plants so protected, originated with Prof. W. W. Tracy, of Detroit, Mich. in 1887. The material (manilla paper) which he used, however, was unsuitable for the purpose and the results of his experiments were unsatisfactory. In 1889 Prof. E. S. Goff of Wisconsin (7) substituted tarred paper for the material used by Tracy, with complete success. Goff also devised an efficient tool for cutting the discs expeditiously; the latter were about three inches in diameter, hexagonal in shape, with a slit extending from one angle to the centre and with a star shaped cut also in the centre.

Since Goff proved his method of protecting cabbages and cauliflowers by means of tarred discs to be thoroughly practical it has been widely adopted in America by commercial growers and others. The method has also been tested at almost all the Agricultural Experiment Stations in the United States and Canada in those districts where serious infestations of Cabbage-Maggots occur: with one or two exceptions the resulting reports have been entirely favourable.

The following authors have published reports of successful trials of tarred felt discs: Slingerland (11), Smith (12), Britton and Walden (3), Caesar (4), Schoene (8 & 9), Britton and Lowry (2), Gibson and Treherne (6).

On the other hand Schöyen (10), Blair (1), and Washburn (15) obtained unfavourable results in the trials which they made of tarred discs.

In these cases the lack of success is attributed either to placing the discs on the plants too late in the season, or carelessly, or to using tarred paper—which curls—instead of tarred felt.

The greater weight of opinion and evidence is distinctly in favour of the tarred disc method of protection and the majority of the above quoted observers agree that it is practical, economical and effective.

Apparently, however, there are no published records of any trials of this method having ever been made in this country. References in British horticultural literature, and in reports on insect pests, to the use of tarred felt discs are very meagre and not very encouraging.

As numerous complaints of damage and losses sustained by growers owing to the depredations of the Cabbage-Maggot are continually being received, together with requests for information how to deal with the pest in question, the desirability of testing here the method which has proved so successful in America thus becomes apparent.

During successive seasons in my own garden I have lost considerable numbers of cabbages and cauliflowers owing to attacks of the Cabbage-Maggot and I had already decided to test the American discs when Dr A. D. Imms suggested that I should test them on a fairly large scale in a local market-garden. Arrangements were therefore made with a market-gardener to rent from him a piece of land and the work was commenced in the spring of 1916.

Description of the Experiments.

The tarred felt discs were obtained from the United States where they are regular articles of commerce. They were not the hexagonal form usually figured and described but they were square, being $2\frac{1}{2}$ inches each way with only two slits—a long slit extending from the middle of one edge to a point half-an-inch beyond the centre of the disc, and a short slit three-quarters of an inch long crossing the long slit at right angles in the centre of the disc (Fig. 3).

Two separate pieces of land situated at Northenden, Cheshire, were rented from Mr Chas. Heywood, a market-gardener, who undertook to prepare the land and perform the necessary operations of cultivation, planting, etc. The tests were made on both cabbages and cauliflowers and the two plots of land were about sixty yards apart. The land on which the cabbages were planted was under cauliflowers in 1915 and the crop then suffered severely from maggot attack, from two to three-fifths of the plants being lost owing to this cause. This piece of land was selected in order to ensure, so far as possible, a heavy infestation of maggots.

A heavy dressing of well-rotted farm-yard manure was spread over the land towards the end of April; this was then ploughed under, the land afterwards harrowed down and then rolled in order to consolidate the soil—which is very light in texture—and to render conditions favourable for placing the discs as flat as possible.

(*a*) *With Cabbages.*

The cabbages (variety Leeds Market) were planted out on May 1st and the discs were placed in position on the following day. Altogether 816 cabbages were utilised for this experiment; they were planted in eight rows, each row containing 102 plants: disced and undisced rows alternating with each other. The spring was a particularly good growing season and the plants made excellent growth. On June 18th, some of the unprotected plants first showed signs of maggot attack and on June 23rd a count of the plants was made. None of the protected plants showed signs of attack but thirty-one of the unprotected plants —equal to 7·6 per cent.—exhibited the usual signs of severe infestation. The attack was very evenly spread over each of the four undisced rows: the numbers of attacked plants in each row being nine, seven, seven, and eight respectively.

On July 8th the cabbages were again counted when the percentage of attack had increased to 13·2; fifty-four out of the total number of 408 unprotected plants being severely attacked, whilst only one of the protected plants had succumbed to maggot attack, i.e. less than 0·25 per cent.

Several of the infected plants were taken up for examination and in all cases the original fibrous roots were absent, having been destroyed by maggots; in some cases the latter were still feeding on the roots and in those cases where they were absent pupae were present in the surrounding soil. In many plants new fibrous roots were developing near the upper portion of the main root and it was evident that the attack caused by the first generation of flies was ended.

The opinion of the market-gardener was that possibly one-third of the infected plants would develop a fresh set of roots and recover from the attack, although the resulting cabbages would be only small.

On July 13th the plants were counted again. No further attack was evident; the cabbages were counted at intervals of seven days until mid-August but no further losses were incurred.

As only one plant was lost owing to maggot attack out of 408 plants protected by the discs it is very strikingly evident that the latter afforded a very complete protection to the plants. The protected cabbages were also larger on the average and slightly earlier in coming to maturity than those in the unprotected rows. A probable explanation of these differences will be mentioned when discussing the results of the cauliflower trials.

Some alterations in the discs themselves were made and the method of placing them on the plants was also varied, as follows. On one row all the discs used were perforated with a small hole in the centre where the two slits cross, in order to obtain a closer fit round the stems of the plants. On another row two discs were applied to each plant, the discs being crossed so that when the slits are forced open by the stem thickening during growth, the soil is not exposed, as it is to some extent when single discs alone are used. Where two discs were used it was surmised that female flies would be absolutely prevented from gaining access to the soil near the plants so protected. As will be seen from the results obtained the single discs gave quite adequate protection and no corresponding advantage was gained by using either perforated discs or double ones to compensate for the additional expense and trouble entailed.

(*b*) *With Cauliflowers.*

The land on which the cauliflowers were planted was similar in character to that on which the cabbages were grown; it was prepared in the same manner and was cropped with sage the previous season. The seedlings (variety Autumn Giant) were planted out on May 27th and the discs were placed round the plants immediately after planting. The latter were planted in four rows, each row containing 233 plants, i.e. 932 plants altogether. The protected rows alternated with the unprotected ones: rows I and III being protected, rows II and IV were unprotected.

The first count was made on June 23rd with the following result. On row I adjoining the field (see Fig. 1) no plants showed signs of maggot infestation; row II, sixty-two plants attacked; row III one plant infected; row IV, ten plants had succumbed.

Altogether seventy-two of the 466 unprotected plants were attacked, equal to 15·4 per cent., whilst only one of the protected plants was attacked up to that date.

On July 4th the plants were again counted when considerably further losses had occurred; a photograph (Fig. 1) was taken on this date.

The results of the countings are given in tabular form; a table showing the results obtained with the cabbages is also given for comparison.

No further cabbage plants were destroyed by maggots on this plot after July 8th. 768 cabbages, out of a total of 816 planted, had been

cut and marketed up to the end of September. This number agrees closely with the number of plants (761) unaffected with maggots on July 8th. A few plants had evidently recovered and made marketable plants by the end of September, as predicted by the market-gardener.

Table showing the numbers of Cauliflower plants destroyed by Cabbage-root Maggots when protected by tarred felt discs, and when unprotected. Each of the four rows contained 233 plants.

Dates when counted	Row I protected	Row II unprotected	Row III protected	Row IV unprotected	Results in percentages: protected rows (I and III)	Results in percentages: unprotected rows (II and IV)
June 23	0	62	1	10	—	15·4
July 4	0	132	7	56	1·5	40·3
July 15	5	141	10	85	3·2	48·5
Aug. 5	11	174	13	120	5·1	63·0

Results with Cabbages. Each row contained 102 plants.

Dates when counted	Rows II and IV protected	Rows I and III unprotected	Rows VI and VIII protected	Rows V and VII unprotected	Results in percentages: protected rows II, IV, VI, VIII	Results in percentages: unprotected rows I, III, V, VII
June 23	0	16	0	15	0	7·6
July 8	1	33	0	21	0·2	13·2

The cauliflower plants were also counted on July 8th but no further losses had occurred since the previous count (July 4th). The effect of the infestation produced by the first brood of flies had reached its maximum about this date.

From July 15th onward the losses increased very considerably as will be seen by reference to the table. This increase was attributed to a secondary attack of maggots produced by the second generation of flies. At this period also a spell of three weeks of very hot, rainless weather prevailed; these weather conditions rendered the cauliflowers more susceptible to maggot attack and less able to recover therefrom.

On August 12th the plants were again counted but no further losses were observed; on August 16th some of the cauliflowers were ready for cutting and counting was discontinued.

The results obtained prove very conclusively that the tarred felt discs are a very effective means of protection from cabbage maggot attacks. Only twenty-four cauliflower plants were lost out of a total number of 466 protected by discs, whereas 294 plants were lost out of

the same number of unprotected ones. A net gain of 57·9 per cent. was thus effected by using the protective discs.

The results obtained with the cabbages were even more striking as only one plant was lost out of 408 protected by the discs. The infestation, however, was not nearly so severe in the cabbages as in the cauliflowers. Only 13·2 per cent. of the unprotected cabbages were lost as against 63·0 per cent. of the cauliflowers.

Market-gardeners, and gardeners generally, hold the opinion that cauliflowers are much more liable to be destroyed by maggot attack than cabbages, and the results obtained in these trials give strong support to this view. Whether it is a fact that cabbage-root flies are more strongly attracted to cauliflowers than they are to cabbages, or whether the former plants succumb more readily to maggot attack than do cabbages, remains a subject for further enquiry. The above results clearly show that, on similar land, cauliflowers suffered much more severely from root-maggot infestation than cabbages. The only factor that differed in the two cases, was that the cabbages were planted out a month earlier than the cauliflowers, and consequently were well established, and growing vigorously, before the maggot attack became severe.

Gibson and Treherne, who discuss very fully this question of the relative susceptibility of cauliflowers and cabbages to maggot attack, make the following statement:

"We consider that the supposed susceptibility of cauliflowers over cabbages is probably due to the lesser vitality of the former plants."

During a period of seventeen days between June 25th and July 11th, 1915, at Agassiz, B.C., 1418 eggs were deposited on six cabbage plants, while only 1038 were laid on six cauliflower plants; these results lead them to believe that no special choice exists on the part of the fly to deposit on cauliflowers over cabbages. On the other hand, in the table given on p. 26 of their bulletin, they state that 6602 eggs were laid on twelve cabbage plants during 133 days, an average of 550·1 per plant, equal to four eggs per plant per day. On six cauliflowers 5515 eggs were laid during 117 days, an average of 919·1 eggs per plant, equal to seven eggs per plant per day; i.e. nearly twice as many eggs were laid on cauliflowers as on cabbages. Although the periods of time were not coincident—the countings of the eggs on the cauliflowers being made later in the year than in the case of the cabbages—the results would appear to indicate that the flies were more strongly attracted to the cauliflowers than to the cabbages.

Precautions to be observed in applying the discs, and other remarks.

In order to obtain the best results from the use of tarred felt discs it is advisable that the soil should be in a fine friable condition when the discs are placed in position, and further, that the plants should not be placed in depressions of the soil but if possible on a slight ridge. If the soil is lumpy the discs will not lie flat on the ground and the female flies will then be able to crawl underneath the discs to deposit eggs. If the discs are placed below the soil level they are liable to become covered with soil after rains, which renders them less efficient. It is also of the utmost importance to place the discs round the plants immediately after planting out, if this operation is performed later than the first week of May. In the district around Manchester, eggs of the cabbage-fly were found on May 14th in 1915, and on May 18th in 1916; these were the earliest dates they were found in those seasons. Probably in the South of England they would be found much earlier, and in the North somewhat later. On seedlings planted out earlier in the year, it is not so important to place the discs in position immediately after planting, although there may be advantages to be gained by doing so.

Several of the observers, who have tested the tarred felt discs, draw attention to the fact that protected plants are usually larger, and mature earlier, than unprotected ones. These differences are rightly attributed to freedom from root injury owing to the absence of maggots on the roots. Another possible factor, which contributes to this result of increased size and early maturity, may be referred to here. If the soil underneath the discs is examined on hot days, during the first two or three weeks which follow the planting out of the seedlings, it will be found in a moist condition, whereas the soil round the unprotected plants will be dry. The disc, by preventing evaporation from the surface of the soil, conserves the moisture in the soil directly beneath it. The plants are thus enabled to recover more quickly from the effects of transplanting, and the conserved moisture also helps them to withstand better the effects of prolonged drought. More especially is this the case with regard to cauliflowers, which suffer more from drought than cabbages.

The question is frequently asked whether the discs, owing to the smell of the creasote with which they are impregnated, deter flies from

laying eggs. Whilst the creasote may exercise a deterrent influence on the flies for a short period of time after the discs have been placed round the plants, this influence—supposing it to exist—soon disappears. Frequently eggs may be found both on the surface of the discs, and also underneath them: a fact which tends to disprove the idea that flies are deterred from laying eggs owing to the emanation of odours from the discs themselves. The chief function of the disc is to act as a mechanical obstacle to the efforts of the flies to deposit eggs in the soil near the plant.

Growers have also asked for information regarding the action of the discs on slugs. No definite experiments have been made with the object of determining the effects of the discs with regard to slugs. It is worthy of note however that, in the row of 233 cauliflowers adjoining the field of clover (see Fig. 1), only two plants were definitely proved to have been injured by slugs. As numerous slugs were observed in wet weather along the edge of this field, it would appear probable that they do avoid the discs. Occasionally a slug was found sheltering beneath a disc, but this was where the ground was lumpy and spaces were present underneath the disc. If the discs are pressed closely to the soil, no accommodation would remain for slugs, and they would thus be prevented from crawling underneath.

Several market-gardens were visited during the summer months in order to determine the relative proportion of plants destroyed by maggot attacks on different types of soil. An interesting fact was observed in a market-garden situated about two miles from the one where the above described experiments were conducted. Although I examined hundreds of cauliflowers there, it was difficult to find a single plant infected with root-maggots. The soil is a heavy clay loam—the owner described it as a good strong soil—and the gardener attributed to this fact his immunity from root-maggot attacks. He also uses gas lime abundantly, having a strong belief in the beneficial effects of this substance against root-maggots and pests generally. The contrast between the two cauliflower plots, at the end of July and early August, was very striking. On the one plot, where the soil was light in character, from fifty to sixty per cent. of the cauliflowers were destroyed by maggots: on the heavy soil, hardly a single plant, in three or four hundred, was lost from this cause. All the growers, with whom I discussed the matter, agreed that cauliflowers and cabbages are more liable to become infested with root-maggots when grown on light soils, than they are when grown on heavy soils. Various reasons were given in support of this opinion, and

without attempting here to discuss this aspect of the question, I may say that all the evidence obtained during the summer in this connection supports their view.

Trials of the discs have also been made with winter-greens and flowering broccoli. Very little advantage has been apparent with regard to these plants up to the present time (end of September). Probable reasons for this fact being that the intensity of attack of the second brood of flies was declining about the time when planting out took place, and also because at this period (end of July and early August) the greatest numbers of *brassica* plants are growing in the fields; consequently the numbers of eggs laid near any single plant are not likely to be so numerous as when fewer plants are in the fields and gardens, as is the case earlier in the season.

As stated above, tarred felt discs are regular articles of commerce in America, where they are largely used by growers. The discs used in these experiments were obtained from A. B. Cowles, 25, S. Water St., Rochester, N.Y.; they cost two dollars per thousand (120 for a shilling) in addition to carriage. In this country at the present time tarred felt discs are not obtainable commercially. Various kinds of materials, however, have been tested this summer and arrangements are in progress whereby, it is hoped, suitable discs will be purchasable at a reasonable price in England. Growers who suffer losses from root-maggot attacks will thus be enabled to test for themselves the value of this method of protection.

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DESCRIPTION OF PLATE XIV

Fig. 1. Photograph showing appearance of the cauliflower plot on July 4th. Rows I and III (counting from the field on the left side of the photograph) were protected with tarred discs, rows II and IV were unprotected. The remaining rows of plants were not utilised in these experiments.

Fig. 2. Photograph of a brussels-sprout plant showing a disc in position.

Fig. 3. Outline figure of a disc; actual size.

Fig. 1

Fig. 2

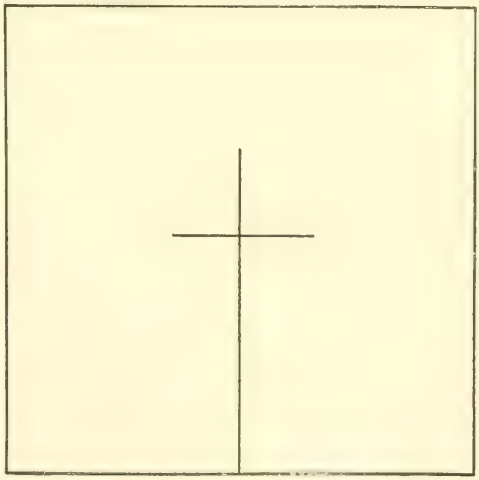

Fig. 3

ON THE RESISTANCE TO FUNGICIDES SHOWN BY THE HOP-MILDEW (*SPHAEROTHECA HUMULI* (D.C.) BURR.) IN DIFFERENT STAGES OF DEVELOPMENT.

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(With Plate XV.)

In the testing of the resistance of any fungus to a chemical substance both the environmental conditions and the stage of growth of the fungus are, obviously, important factors.

We know, *e.g.* from Clark's[1] experiments, that the spores of a fungus when supplied with nourishment in the form of a decoction of sugar-beet show a greatly increased power of resistance to the toxic effects of copper sulphate. With *Œdocephalum albidum* the lethal strength for spores in water is ·0013 %, whereas with spores supplied with nourishment it is 0·1 %,—about 80 times as strong.

In a recently published paper[2] dealing with the fungicidal action of certain chemicals, principally sulphides, attention was drawn to the fact that *data* as to the behaviour of spores placed in a fungicide have little practical value as indicating the strength at which the same chemical will be fungicidal when used against the growing fungus on the plant. It was pointed out that while Foreman[3] found that a 0·16 % solution of caustic soda prevents the germination of spores of *Botrytis cinerea* and *Sphaerotheca mors-uvae*, experiments have shown that a 0·3 % solution does not kill well-developed patches of the conidial stage of *S. Humuli*.

In all our spraying experiments with "powdery mildews" (*Erysiphaceae*) carried out during 1914 and 1915 we were careful to select for spraying well-developed patches of mildew showing dense clusters of conidiophores bearing chains of conidia—a stage of development

[1] *Bot. Gazette*, Vol. XXXIII (1902).

[2] J. Vargas Eyre and E. S. Salmon, "The Fungicidal Properties of Certain Spray-fluids, *Journ. Agric. Science*, Vol. VII, 473 (1916).

[3] Foreman, F. W., "The Fungicidal Properties of Liver of Sulphur," *Journ. Agric Science*, Vol. III, 401 (1910).

which may be briefly described as a "powdery" patch. This stage was chosen both in order to make the experiments as strictly comparable as possible and on the assumption that in such a stage of development—where the fungus has a well-developed mycelium furnished with very numerous haustoria—the resistance to the fungicidal properties of the chemical would be at its highest.

During experiments this season (1916), however, facts have been observed which show that the mildew when in an earlier stage of development on the host-plant is more difficult to kill. Our suspicions of this fact were first aroused by the occurrence of minute tufts of conidiophores at fresh places on the leaf two or three days after the spraying had taken place. These fresh little patches of mildew could not have been due to re-infections subsequent to the spraying, since a conidium takes about five days from infection to develop mycelium and conidiophores. The point was then studied more closely by means of hop-leaves inoculated with conidia of the hop-mildew. It was found that very soon after being inoculated the young hop-leaf, when rapidly growing, shows small raised "blisters" or "humps" at the places where it has been infected. At first the raised blister is green all over, and shows no trace to the naked eye of a white mildew, although usually, under a lens, a few delicate hyphae may be seen radiating from the centre of the "hump." After a day or two, each "hump" shows a whitish growth beginning to spread over its surface, due to the formation of branched mycelial hyphae, and soon afterwards the first conidiophores appear. An exactly similar appearance may be found on a young Rose-leaf when attacked by the Rose-mildew (*S. pannosa*).

Spraying experiments on hop-leaves bearing numerous "humps" showed that the mildew in this very early stage of development has a greater power of resistance to sulphide solutions. Thus in experiments it was found that a certain stock solution of ammonium sulphide when diluted 1 to 100 with water was completely fungicidal to the hop-mildew when in its "powdery" conidial stage; this solution did not, however, prevent the youngest stage of the mildew, described above, from developing and forming conidiophores. At double the concentration however, the sulphide solution in question became fungicidal for this earliest stage of development.

The details of two experiments may be given here. In *Exper.* 1 the fungicide used was a certain concentrated solution of ammonium sulphide diluted 1 to 100 with water (and containing 1 per cent. soft soap). Three seedling hop-plants were used, and the leaves on each which were sprayed bore the hop-mildew in the stages of development noted below:

plant *a*: leaf at 5th node (from apex) bearing numerous "powdery" patches.
„ 4th „ bearing "humps," showing a few very young sterile hyphae.
plant *b*: „ 5th „ bearing numerous "powdery" patches.
„ 4th „ bearing "humps," showing a few young sterile hyphae.
plant *c*: „ 5th „ bearing numerous "powdery" patches.
„ 4th „ bearing green "humps" which showed under a lens no signs of any hyphae.

All the plants bore at the respective nodes two leaves, each with the mildew in the same stage of development, and one leaf at each node was left unsprayed as a control. The spraying (using an atomiser) was carried out on June 6. By June 10 it was apparent that on all the sprayed leaves where the mildew had been in the "powdery" conidial stage the ammonium sulphide solution had almost or quite killed the fungus; in every case, however, the leaves which bore the humps showed the mildew in a further stage of development. On plant *a* there were numerous tiny tufts of conidiophores appearing from the "humps"; on plant *b* there were two such tufts of conidiophores, and on plant *c* nine such tufts. By June 17 the original "powdery" patches on the three sprayed leaves were all dead and in many cases semi-obliterated; the control leaves all bore very numerous almost continuous densely powdery patches. The sprayed leaves with the "humps" were as follows: *a*, ten very small though densely powdery tufts had developed over the "humps"; *b*, seven small densely powdery tufts of conidiophores had developed where the youngest "humps" had been,—where the "humps" had shown, at the time of spraying, mycelial hyphae, the fungus had been killed; *c*, twelve tiny powdery tufts of conidiophores had developed over the "humps." In every case the control leaf showed much more numerous and much larger densely powdery patches of conidiophores. It was clear from this experiment that the ammonium sulphide solution used was fungicidal for the mildew in the "powdery" conidial stage, but was not fungicidal for the mildew in its earliest stages of development, although it appreciably checked its growth.

In *Exper.* 2 the fungicide used was the same concentrated solution of ammonium sulphide diluted 1 to 50 with water (and containing 1 per cent. soft soap). The seedling hop-plant used bore at the 5th node (from the apex) two leaves, each bearing very numerous "powdery" patches of mildew, and at the 4th node two leaves, each bearing from thirty to forty "humps," only a few of which showed young mycelial hyphae on their surface. One leaf at each node was sprayed on June 6; the remaining leaves served as controls. On June 10 the "powdery" patches on the sprayed leaf were all dead and semi-obliterated; the "humps" on the sprayed leaf showed no signs of any living mildew. On June 12 the control leaf at the 5th node bore numerous densely powdery patches, while the sprayed leaf showed no signs of any living mildew; at the 4th node the sprayed leaf bore about thirty "humps" with no trace of mildew developing from them, while the control leaf bore over thirty "humps" each of which now bore a small densely powdery patch of conidiophores. On June 20 the sprayed leaf at the 4th node was of a healthy deep green colour and still showed the original "humps" but no trace of mildew occurred anywhere on the leaf; a photograph of this leaf as it appeared at this date is reproduced in Plate XV, fig. 1. The control leaf at the 4th node now bore densely "powdery" patches covering over the original "humps"; a photograph of this leaf is shown in Plate XV, fig. 2[1].

[1] I am indebted to Mr H. Wormald for taking these photographs.

Further, some evidence has been collected showing that the age of the mildew even when in the "powdery" conidial stage is a factor of importance. The older conidial patches have less power of resistance to the soluble sulphide spray than the young patches.

The explanation of the difference in resistance to fungicides shown by the earliest and the later stages of development cannot be given until further *data* are available. It would appear that to some extent the age and condition of the host-leaf are concerned; the mildew has less resistance in its conidial stage when on an old hop-leaf than when on a vigorous young leaf. But that this is not the whole explanation is shown by the fact, mentioned above, that on one and the same leaf a sulphide solution will kill the mildew where present in its well-developed conidial stage and fail to kill it in its youngest stages of development. It is possible that the earliest stage of development is more resistant because the first-formed haustorium from the appressorium of the germ-tube has more vigorous powers of resistance than the later-formed haustoria, or because the appressorium offers powers of resistance; on the other hand it may be that the vigorously growing conidial stage is less resistant through the formation of thinner-walled hyphae, or that the great number of haustoria present allows the fungicide to reach more easily the epidermal cells of the host-plant and cut off the food-supply.

On the old hypothesis that the sulphide solution acts fungicidally by virtue of the sulphur deposited, it could be held that the "powdery" conidial patches are killed because a sufficient amount of the sulphide solution is collected round them to give the requisite deposition of sulphur, while this would not take place in the case of the earliest stage of development when there are few mycelial hyphae and no conidiophores. We are unable to accept this explanation, however, since our work of last year has led us to the conclusion that with regard to this class of fungicides the sulphides contained in solution act as such and not by virtue of the sulphur deposited.

Whether it will be more economic to attempt to kill a "powdery mildew" by spraying at a strength lethal to its earliest stage of development, or to wait until the mildew has developed into the less resistant conidial stage, must be decided by future experiments in the laboratory and field. It is clear, however, that in the treatment of "powdery mildews" generally this difference of susceptibility at different stages of development must be kept in mind as a new factor of importance.

Fig. 1

Fig. 2

OBSERVATIONS ON THE LARVAL AND PUPAL STAGES OF *AGRIOTES OBSCURUS*, LINNAEUS.

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(With Plates XVI and XVII and 1 Text-figure.)

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1. Historical.

Although the larval stage of *Agriotes obscurus* has undoubtedly been of economic importance for a long period, yet there does not appear to have been a good description of it until Westwood published a brief account in 1839. Marsham in 1808(12) had figured a larva which he considered to be a stage of *Elater segetis* (= *Agriotes lineatus*), but the figure is too indefinite for accurate determination. Furthermore, he says he was unable to breed out the perfect insect, and was relying on Bierkander for his information.

Westwood(18), however, describes the larva of *Agriotes obscurus* and figures the antenna, the under side of the head and prothoracic segments,

and the first maxillae and labium; his figures are too small to be of much utility. He further notes that the larva had been figured previously by De Geer and Bierkander; but points out inaccuracies in the figures of these authors, that seem to indicate faulty identification.

Chapuis and Candèze in their work published in 1855(3) do not describe the larva, but merely quote references to the writings of Marsham and Westwood. In 1860, Curtis published in his work on *Farm Insects*, an account of Elateridae of importance to agriculture; he did not describe the larva of *Agriotes obscurus*, and considered the larva of *A. lineatus* to be the common and important species.

Schiodte(16) defined larval characters of the genus *Agriotes* in 1869, and described those of *A. lineatus*. He figured the left mandible, as well as the eighth and ninth abdominal segments. No mention is made of *A. obscurus*. Perris in 1878(14) described many larvae of Elateridae; but he merely quotes references to Marsham(12) and Westwood(18), and does not add any description of *A. obscurus*. Beling(2), in a full account of the metamorphoses of Elateridae, published in 1883—4, describes the larva of *Agriotes lineatus* very completely, and states that he could only detect slight differences between the larvae of *A. lineatus* and *A. obscurus*. Finally, the subject is brought up to date by Xambeu(20) in 1912 and 1913 by his work on the life histories of Elateridae. He adds little to our knowledge of *Agriotes obscurus*, merely referring to Beling (*op. cit.*) and adding a few descriptive remarks. It has been somewhat difficult to trace literature definitely referring to *Agriotes obscurus*, owing to the long established custom of terming Elaterid larvae under the collective title of "wireworms," without reference to any definite species. This, together with a number of cases of inaccurate identification in the older literature, has often rendered the information of doubtful specific value.

2. Material and Methods.

The idea of studying the life history of certain Elateridae, whose larvae are known as "wireworms," was first suggested to me by Dr Imms, to whom my thanks are also due for advice given from time to time. The first problem requiring solution, was the determination of the exact species of which the common wireworm forms the larval stage. With this intention, the material was purposely obtained from as wide a range of localities, and under as different conditions (of soil, crop, etc.), as possible. The area covered was mainly in Cheshire and around

Manchester, while a fair number of specimens were taken in various districts of Mid-Lancashire and North Staffordshire. Wireworms were taken in soils growing a variety of crops, but the most abundant and sure source of specimens was found to be the potato crop; a visit to a potato field rarely proving a disappointment. Pastures, especially those that had been down for a long period, proved a good source for material. They were, however, only utilised until sufficient observations had been made, owing to the lengthy examination required and the uncertainty of results. The material when collected was put into fairly deep tin boxes, loosely packed with damp soil. The larvae were examined individually under a binocular microscope in the laboratory, being measured and roughly classified into sizes. They were placed, after examination, in plant pots previously prepared. As this type of breeding cage proved to be useful, no trouble from disease having been experienced, some further details may be of interest. The drainage hole of the plant pot was covered with a circular piece of fine wire gauze strengthened by having a strip of zinc soldered round the edge. The gauze was roughly two-thirds the width of the pot; so, no matter how it slipped, the gauze would still cover the drainage aperture and prevent the escape of any larvae. The top of the flower pot was covered over by a perforated zinc plate, having a flange of about an inch in depth. The flange and the weight of the zinc were sufficient to keep the pot covered securely, even in the most boisterous weather. The vessel was filled first with small pieces of broken pots, and then with earth in the usual manner; a potato was placed about half way down in the soil to provide food for the larvae. The pots were buried out of doors in the ground, up to the level of the soil inside the pot. The larvae lived quite healthily in these pots, and no sign of disease was ever observed. For experiments requiring a greater depth of soil, two ordinary three-foot drainpipes were used; the narrow end being capped with cement, which was sloped inside to a central aperture covered by wire gauze imbedded in the cement. These drainpipes were very useful, but were too weighty to handle easily. They were handicapped by being buried in stiff clay soil, which prevented good drainage and rendered the drainpipes liable to become waterlogged. Buried in soil of a more open texture and better drainage capacity, they would be quite free from any disadvantage, save that of weight.

Throughout this work, the endeavour was to keep all the conditions as closely approximating to nature as possible.

The greater part of the laboratory work has been carried out in the Department of Agricultural Entomology of Manchester and I am also much indebted to Professor Hickson for affording me various facilities. Acknowledgment has already been made to Dr Imms. I am also indebted to Mr J. T. Wadsworth for the photograph of the pupal cell with the pupa *in situ*. Mr J. C. F. Fryer of the Board of Agriculture has kindly given me information as to distribution, etc., of the common wireworms. The work has been carried out during my tenure of a Board of Agriculture studentship and completed during the following year.

3. Notes on the Biology of the Larva.

The larval stages of Elateridae are practically ubiquitous, occurring in all types of soil. The larvae of *Agriotes obscurus* have been found most commonly in the lighter types of soil, particularly loams rich in organic matter. The range of soils that they inhabit is extraordinarily varied, including the grades between fairly stiff clay to light loam. They are practically always to be found in pastures, even when on clay soils, though in such a case they are much less plentiful. In Cheshire and Lancashire the common wireworm is the larva of *A. obscurus*, as was demonstrated by breeding experiments. This seems to be corroborated by the fact that Newstead records adult *A. obscurus* (when in a state permitting of identification) far oftener than that of *A. lineatus*, as being the common species taken as food by wild birds over the same area. Very probably, *A. obscurus* is the common species in the north of England; while *A. lineatus* seems to be the most common species further south, though *A. obscurus* and *A. sputator* are not far behind in point of numbers. An interesting feature with regard to *A. obscurus* is, that the occurrence of its larvae seems to be, in some way, closely connected with the presence of organic matter in the soil. The larvae are most abundant in soils containing a large percentage of humus, due either to the peaty nature of the soil, or to heavy manuring. The same thing was found in cottage gardens, which are usually very rich in organic matter. This point will be considered later in connection with the feeding habits.

The larvae of *A. obscurus* were found in what may be roughly described as three stages. These stages were, of course, not sharply defined, but merged into one another. They may be given approximately:

Small stage (1) 7 mm. long by 0·75 mm. broad.

Medium stage (2) 10—15 mm. long by 1·0—1·25 mm. broad.

Large stage (3) 17—23 mm. long by 1·5—2·0 mm. broad.

It should be stated that no living specimen was ever found to be more than 21 mm. long, and that 19 mm. is about the average length, taking a number of specimens into consideration. The 23 mm. specimen was an extended spirit example. After about a year, the small stage was no longer present, and only medium and large stages could be found in the plant pots. According to most authorities the larval period is supposed to last five years. From observations on the length and rate of growth, I am inclined to place the period at four years rather than five. The small stages, taken in one year from July to October, varied much in size. It was found, after a couple of months (November—December), that a number of these apparently small specimens were really of medium size. It appears from this that the breadth is a safer criterion than the length (which is difficult to ascertain on the living larva). Judging from the size of specimens taken from July to October, and also from the fact that the adult beetles are common from April to June, it may be assumed that the newly hatched larva commences its free existence in July. Again, there are three larval moults, performed in the usual manner, by splitting the chitinous skin along the thorax, and leaving the old exo-skeleton behind. From these facts, and from the rate of growth, it may be assumed that the larva is full grown in three years. Full size larvae are found actively feeding up to October; but these same larvae apparently do not feed again (at any rate on vegetation), and pupate the next summer. It is thus probable that there are three stages limited by the three moults, and occupying three years. Then there is a period of active feeding, followed by a quiescent condition terminating in pupation, all this taking place in a year. It was found that of the large larvae (17 to 23 mm.) a number fed through the winter, while some were not feeding after October, and were not lethargic; these latter were broader than were the former. This would appear to support the above theory. If the life of the larva is one of five years, then it would seem that the early (small) larval stages would continue over a longer period; this is not confirmed by observations. The rate of growth was found to be of such a uniform character as to suggest that the curve of growth is fairly continuous rather than irregular. It seems to the author that the period of growth is not five years, a period given since Bierkander, but probably requires four years from the egg to pupation. The early (small) stage larvae were never found feeding. It is stated that they

feed on organic matter in the soil. The medium and large stages were almost invariably observed feeding (with the exception mentioned above in the case of the larger stage), and it seems that they feed continuously, save in the months of December to February inclusive. This observation was obtained from regular examination of potatoes placed in the breeding pots. Feeding is usually active by March and seems to decrease at the end of November. It is very difficult to starve these wireworms. Of 10 large wireworms placed in sifted soil without food November 3rd, 1914, nine were present January 22nd, 1915, and seven on March 8th, 1915. The three missing ones had probably been eaten by the others, as traces of the skins were found. These seven larvae had thus existed in soil free from any vegetable matter visible to the naked eye for 125 days; of the seven larvae only one remained on May 4th, 1915. In a duplicate experiment under similar conditions, 10 larvae lived from November 3rd, 1914, to February 11th, 1915, i.e. 100 days. The larvae will tolerate a large amount of moisture, and will exist for at least a week in water-logged soil. On the other hand, wireworms kept in soil not supplied with water and allowed to dry up will die after a couple of days. It is hard to explain their independence of vegetable growth (such as pasture grasses and various crops) for food, in any other way than by assuming that they feed on the organic matter in the soil. This would help to explain the usual occurrence of these larvae in the lighter soils plentifully supplied with organic matter. In several cases, small and medium sized larvae were found buried in the farmyard manure under growing potatoes. It could not be said definitely that the larvae were feeding, but the condition of the mouth parts seemed to indicate that they were.

The usual depth at which these wireworms are found in the soil varies from one inch in pasture, to eight or nine inches in potato fields. In winter they bury themselves much deeper, and were found as much as two feet deep in the large drainpipes previously described.

The larvae bury themselves in soil surprisingly quickly, though their rate of progress on the surface is comparatively slow.

Eighty-three larvae had buried themselves at least two inches deep in soil, of about the same consistency of that in a potato field, in less than twenty minutes. On being exposed to light, they immediately travel in the opposite direction to the source of the light. They travel slowly, but fairly easily on an unpolished surface, and often progress carrying the abdomen bent in a slight curve to one side. This is one of the difficulties that hinder accurate longitudinal measurement.

4. Description of the Larva.

The full-grown larvae of *A. obscurus* measure about 19 mm. × 2 mm. (Plate XVI, figs. 1 and 2). They are semi-cylindrical and their colour varies from pale yellowish white, to yellow brown. The young stages are of a paler colour, which darkens as the larvae become older. The freshly-moulted larvae are easily distinguishable by their pale yellow white colour. The junctions of the body segments are of a slightly darker shade than the rest of the body, and are in addition marked with faint longitudinal striae (Plate XVII, fig. 6). The spiracles are placed on darker chitinised areas. The muscular impressions of the ninth abdominal segment are strongly chitinised dorsally and ventrally, and slightly so laterally. The legs are of a darker brown colour.

The head is approximately as broad as long, with the anterior margin of a dark brown, rounded at the angles. On each side of the head, just behind the antennae, but slightly more towards the median line, is a slightly raised, irregularly rounded, pigmented eyespot. Immediately behind each eyespot is a long slender seta. The antennæ are short (Plate XVII, fig. 10), each consisting of three segments. The first segment is stout, brownish round the base, with a clear area round the summit. The second is dark brown, longer in proportion to its breadth, narrower than the preceding segment, and somewhat enlarged on its outer and upper margin where it bears two stiff upright hairs. The third segment is dark brown, about one half the dimensions of the second segment, and bears two terminal lobes which are slightly lighter in colour than the second segment. The lobes of the third segment, when seen from above, consist of a long narrow lobe (almost as long as the second segment) flattened at the apex, and a smaller lobe placed ventrally to the larger lobe and nearly half as long as, and a little thicker than, the latter. The third segment bears, in addition, two setae. The head region bears two pairs of setae on the lateral margin, placed near the anterior and posterior edges. No attempt is made to discuss the homologies of the head region.

The mandibles (Plate XVII, fig. 7) are of a dark brown colour, irregularly flattened dorso-ventrally, and sickle shaped. The apex is somewhat blunt, and just below this is a blunt tooth on the inner margin. This blunt tooth is common to the genus *Agriotes* according to Schiodte(16). A second tooth that is more pointed than the former, projects nearly half way down. Just below, is a well-marked process (*la*) projecting inwards which is the "lacinia mobilis." Mangan(11) in describing the

mandibles of *Periplaneta australasiae*, quotes Hansen (*Ann. Mag. Nat. Hist.*, 6, vol. XII, 1893), as recording the "lacinia mobilis" as occurring in certain Coleoptera. The mandible articulates with the head, by means of the condyle (*co*) and the ginglymus (*gi*).

The first maxillae (Plate XVII, fig. 8) are strongly chitinised around their outer margin, and are of a uniformly brown colour. They possess a distinct cardo (*ca*) into which the chitinised margin continues, swelling into a small dark area. There is no definite distinction between the stipes (*st*) and the terminal lobe of the maxilla. The stipes bears, on its outer margin, two long thick setae and three shorter ones, all close together, and placed rather more than half way up. The maxillary palp (*mp*) consists of four joints, of which the first is broad in comparison with its length, the second nearly as broad but longer, the third narrower and shorter; all three segments are brown, darker at the edges, with clear areas at their summits. Of the three segments, each bears setae; the first, two irregularly placed; the second, two at the summit and two on the outer margin; the third, two on the summit. The fourth segment is short, rounded terminally, and of a light brown colour. A galea (*ga*) and lacinia (*la*) are present in each first maxilla. The galea is short, and composed of two joints; both being about as long as the first two segments of the maxillary palp. The terminal segment bears four short, slightly-twisted setae. The lacinia is not plainly differentiated from the galea, and bears a large number of closely-packed, slender, waving setae.

The labium (Plate XVII, fig. 9) consists of the basal mentum, which is brown, and slightly chitinised at the lateral margins. It bears, at the margin on each side towards the base, a long curved seta, and another on each side towards the apex of the labium.

The mentum articulates with the palpiger, which bears a two-jointed palp on each side. The palpiger is shield-shape, and of a dark brown colour, darker at the sides, and lighter in the median area, and with a clear space across the anterior margin. It bears two long setae placed anteriorly. The first joint of the labial palp swells out towards its extremity, and bears a whorl of small setae, commencing about half way up from the inside, and progressing outwards and upwards. The second joint of the labial palp is rounded, and appears to possess a flattened, pale area at the tip.

Between the two labial palpi is the ligula (*li*) which bears a short seta on each side. The further setae, much smaller than the previous two, are placed at the base of the ligula.

On the underside of the head, adjacent to the first maxillae, is a dark, strongly-chitinised ridge, diverging anteriorly and converging posteriorly. A dark line runs parallel, close to the preceding ridge.

The prothorax has a curved furrow running down each side, and is nearly equal in length to the meso- and meta-thorax taken together. The hairs on the body of the larva are arranged in a definite manner, which seems to be fairly constant. The first eight abdominal sterna each possess three pairs of hairs, arranged symmetrically along the margins of these sterna. On examining the larva in its lateral aspect, it is seen that the first eight abdominal terga, considered up to their mid-dorsal line, possess two lateral pairs of hairs; of which the posterior pair is placed near the margin, a little to the left of the plane of the anterior pairs, which are placed nearer to their margin than the former. Placed mid-laterally, and just over each spiracle (of the first eight abdominal segments), is a pair of short hairs about half the length of the other hairs. In a line with the spiracle and in the same plane as the two posterior hairs, is a single hair of similar length to the paired ones. The ninth abdominal segment bears, on the base of the pseudopodium (or anal papilla), two pairs of short hairs. There are nine pairs of spiracles, the first being thoracic (Plate XVI, fig. 2, *st*), and placed ventrally. The remaining eight pairs are laterally placed on the first eight abdominal segments anteriorly in each segment. The ninth abdominal segment affords a means of distinguishing the genus *Agriotes*; Perris(14) describes the typical anal segment of the genus as follows: "Dernier segment assez-longuement demi-ellipsoidal, terminé par une pointe, ayant de chaque côté, près de la base, une cavité de l'apparence d'un grand stigmate."

This applies truly to the larva of *Agriotes obscurus*, but requires to be added to. The anal segment is terminated by a slight constriction, which swells out into a blunt point, outlined in black (Plate XVII, figs. 5 and 6). The cavity, that Perris describes as being like a large stigma, is not a respiratory opening, but is possibly a muscle attachment[1]. The walls of the cavity are black, and thickened; only slightly at the sides, but strongly dorsally and ventrally. In shape it is oblong, with the anterior and posterior walls rounded. It is placed very high up, and near the base of the segment. The stigmata are oblong, with their dorsal and ventral walls closely apposed, and strongly chitinised; their median line points forward and upwards, save in the case of the meso-thoracic stigmata, where the median line points forward and

[1] Henriksen (8) also speaks of this structure as an "eye-shaped muscular impression."

outward. The ninth abdominal segment is equal to the length of the seventh and eighth segments combined, and possesses a ventral papilla or pseudopodium. This, according to Sharp(17), "represents a body segment and is generally described as being the protruded termination of the alimentary canal." The pseudopodium is round, sloping gradually to meet the eighth segment, and with a sudden slope posteriorly to meet the remainder of its segment. The apex is white and flattened and bears a few short hairs. The pseudopodium is of some assistance to the larva in walking. The three pairs of legs are provided with spines and are dark brown, short and terminating in a curved claw. The legs do not require any special description; the right leg of the third pair is figured in Plate XVII, fig. 11.

5. Description of the Pupa.

Before proceeding to describe the pupa, some details will be given with regard to pupation, etc. The first pupa was found on Aug. 14, 1915, pupation had therefore occurred within seven days, as the last previous examination had taken place on Aug. 7, 1915. At the previous examination it was observed that several larvae were very sluggish, and subsequent events showed that they were preparing for pupation. The first adult *Agriotes obscurus* was observed in a freshly emerged condition on Aug. 30, 1915. This would place the pupal period at from two to three weeks. Observations on other pupae show that this period is correct. Pupae were found up to the end of September, and all the adults had emerged by the 9th of October. Pupation takes place in an earthen cell at a depth in the soil varying from three to twelve inches. The pupal cell is oval and allows ample space for pupation. The average dimensions of three cells was 5 by 14 by 7 or 8 mm. The photograph (Fig. 1) shows a section through a typical pupal cell, with the pupa lying in a characteristic position.

The internal walls of the pupal cell are smooth, and appear to have been slightly cemented together with some glutinous secretion. The cell will stand air-drying without crumbling away, to a greater extent than an artificial cell made of similar soil but not cemented. The method by which the data concerning the pupal cell were obtained is as follows:—the plant pot, taken into the laboratory and the zinc cover removed, was filled level to the top with soil of a known depth, the soil being slightly compressed to produce cohesion. A piece of stout cardboard was placed firmly over the top and the pot then inverted so

as to stand upside down on the cardboard. The cardboard was gently removed, and the soil in the pot loosened by pressing slightly through the drainage aperture. The pot was then lifted up with a twisting movement; a cast of the interior of the pot was left. This cast could easily be examined with regard to the depth of any specimens in the pot, as the depth of soil added just previous to examination was known. With this method, pupal cells in perfect condition were obtained without trouble. The pupa (Plate XVI, figs. 3 and 4)

Fig. 1. Pupa of *Agriotes obscurus* in earthen cell. ×2. (Phot. J. T. W.)

measures up to 13 mm. long by 3 mm. broad, gradually tapering in the abdomen to a breadth of 1 mm. The general colour is white, speckled on the apices of the abdominal segments with small patches of rusty brown colour. The thorax is nearly as broad as long, and swollen. Two spines project from the anterior angle of the thorax, which is provided at the posterior margin, on each side of the middle line, with a small protuberance. The long, spatulate, posterior corners are each provided with a curved thorn-like structure, of a brown colour and pointing outwards. The third to the sixth abdominal segments are

slightly tooth-shaped at their lateral posterior margins. There are nine abdominal segments, of which the ninth is provided with two spines. Each spine possesses a small spur at the base. According to Curtis(6), these spines are movable. The ninth abdominal segment is provided ventrally with a flap, broad at the base, suddenly narrowing half way down the segment, and continuing in a smaller piece with parallel sides, which terminates in two sharp points. Beling(2) describes the pupa of *Agriotes obscurus* in a similar manner to the above, and adds that the segments of the antennae are as long as broad, that the wing cases reach to the middle of the fourth abdominal segment, and that the third pair of legs reach to the middle of the fifth abdominal segment. In the pupa described above, the third pair of legs only just reach on the fifth abdominal segment, and do not stretch half way.

6. Comparison with Larvae of closely allied Species.

The evidence showing that the common wireworms taken by the author in Cheshire and other districts are larvae of *A. obscurus* is as follows:—

All the larvae were examined under a binocular microscope, and were apparently all similar. At this time I was inclined to believe that the common wireworm represented the larval stage of *Agriotes lineatus*. A comparison of my material with Schiodte's figure of the anal segments of *lineatus*(16) did not solve the problem. The larvae undoubtedly belong to the genus *Agriotes*, and agree in general with the figure of *lineatus* given by Schiodte, but differ as to certain details.

Seven larvae at last pupated and the six adults that emerged were *Agriotes obscurus*. This seems to be corroborated by the interesting observations of Newstead (previously mentioned), where the area covered was mainly Cheshire, and part of Lancashire, and the records of adult Elaters devoured by birds give *A. obscurus* far more commonly than *A. lineatus*. As the occurrence of wireworms is admittedly universal, at any rate in this country, and as their attacks are not epidemic, these facts seem to point to *A. obscurus* as being the parent beetle of the larva in question. The identification of the larva provides the next problem.

The author has been unable to procure an undoubted specimen of the larva of *A. lineatus* for comparison, so reference was made to the literature. It was found that there is a scarcity of good accounts of the larvae belonging to the genus *Agriotes*. Some of the older accounts were

incomplete or even wrong, as Perris(14) quotes Bouché (*Naturg.* p. 186), as describing a larva of an *Athous* or *Corymbites* as *A. lineatus*. The best accounts of Elaterid larvae were found in Perris(14), Schiodte(16), and Beling(2), from which sources the comparisons are procured. The distinction between the genus *Agriotes* and that of *Elater* seems fairly clear. The possession of a blunt tooth, on the internal margin of the mandible (Plate XVII, fig. 7), indicates the genus *Agriotes* according to Schiodte, while Beling gives the presence of the chitinous cavity on the base of the ninth abdominal segment as indicating the genus. Beling describes *A. lineatus* and *A. obscurus*, as follows:

(1) Larve, sehr fein und seicht punktirt, fast glatt, blass braunlich gelb. In erde, vorzugsweise auf Aeckern....*Agriotes lineatus.*

(2) Larve unregelmassig seicht gerunzelt, starker und dichter punktirt, auch etwas dunkler als die vorhergehende gefärbt, mit derselben angleichenorten lebend....*Agriotes obscurus.*

Beling further says that the larva of *Agriotes ustulatus* Schall. is similar, but is smaller. He adds that the larva of *A. obscurus* seems more strongly and thickly hairy (punktirt) and wrinkled than *lineatus*; but characteristic differences remain to be found. His description of the *lineatus* larva would apply equally well to that of *A. obscurus.*

The position of the muscle attachments on the anal segment seemed to hold out a hope of identification, as the one illustrated by Schiodte differed in position from those observed by the author. This is unfortunately of little account, as the method of preserving the larva affects the position. If the larva is simply killed by being immersed in alcohol, it usually contracts, and the wrinkling of the skin and the position of the muscle attachment, shown in Schiodte, are obtained. Boiling the larva, and then passing it through different strengths of alcohol, preserve it naturally. Neither the muscle attachment placed so closely to the base, nor the wrinkled skin as figured by Schiodte, is ever seen in living or properly preserved specimens. There is a difference, however, between the pseudopodia. Schiodte figures the pseudopodium proper (as distinct from its base), as exhibiting three divisions, the third probably pertaining to the non-chitinous and fleshy tip. In the larvae examined by the author, there were never more than two (including the fleshy tip). As there is no reason to suppose Schiodte's figure is incorrect, this difference of the pseudopodium must be taken as specific. The mandible figured above (Plate XVII, fig. 7) differs from that of Schiodte.

A number of mandibles (both left and right) were examined and

were found to agree substantially with the figure given. Schiodte, however, figures the blunt tooth near the apex of the mandible as very slight, while the author's figure shows it to be more pronounced.

Furthermore, the position of the spiracle and hairs on the eighth abdominal segment, as represented by Schiodte, does not agree with the condition observed by me in the larvae of *A. obscurus*. These differences are constant in the specimens examined during this work[1]. The only other description to which I have had access is that of the larva of *Agriotes pallidulus* Ill. by Schiodte. The dimensions of this larva, 9 mm. long by 1 mm. thick, and its intense yellow colour, together with its anal segment, which is rounder than those of *A. obscurus* or *lineatus*, prevent confusion with either the larvae of *A. lineatus* or *A. obscurus*.

It would seem, that if the above differences between the larvae of *A. lineatus* and *A. obscurus* do not apply generally, then one is possibly a variety of the other, as it was formerly believed (Curtis, *loc. cit.*, p. 159).

7. Natural Enemies.

It would be natural to assume that the wireworms, on account of their underground existence, are fairly immune from enemies that prey upon them. In spite of this, they are subject to the attack of a number of persistent foes; particularly various species of wild birds. Wild birds are accountable for the destruction of vast numbers of insect larvae. This is especially so during the nesting season, as "practically all birds except doves and pigeons feed their young on an animal diet, whatever may be the character of the food of the adult"(9). Again, as Collinge remarks—"It should be remembered that the nestling season is also that when the destruction of injurious insects is most needed—i.e., at the period of greatest agricultural activity and before the parasitic insects can be depended upon to reduce the pests"(4 and 5). At present, definite information as to the food of nestling birds is somewhat scanty; but according to Collinge, the

[1] Owing to Mr Ford's departure to Rome he was unable to consult Henriksen's paper (8) before he left England. The Danish entomologist mentions that the larva of *A. lineatus* is "brownish yellow, faintly rugose and punctulate, mostly rugose." With regard to that of *A. obscurus* he adds "dark brownish yellow. On each side, between tergum and sternum a pale longitudinal stripe. Punctulate and with few rugae." I may add that the longitudinal stripe is not evident in any of Mr Ford's specimens. Henriksen does not appear to have reared any examples of *A. obscurus* from the larva to the adult. A. D. Imms.

Starling, Thrush, and Blackbird seem to feed Elaterid larvae to their nestlings as usual constituents of their diet. To quote one example—that of the Starling (Collinge, *loc. cit.*)—the food taken to one nest throughout an averaged period of half a day, required 146 visits and included 13 wireworms (species not stated); this was in the middle of May. It is probable that a large number of Elaterid larvae (as well as other pests) are thus disposed of during the nestling season. With regard to the food of adult birds more information is available, and it is possible to give a rough list of birds that are of importance as destroyers of Elaterid larvae, for "the frequent occurrence of these insects, and especially of the parent beetle, is very marked and goes a long way to prove that they form part of the regular food supply of various species of birds"(13).

The most useful in this respect are Plovers (or Lapwings), Gulls, Rooks, Jackdaws and Starlings, all devouring both adult and larval stages of Elateridae. In districts in proximity to the sea, Gulls are very beneficial—they are usually present where land is being ploughed; and they follow the plough, in numbers, searching for insects that are turned up. In one instance (Newstead, *loc. cit.*), forty-five *Agriotes* larvae were found in the crop of a Black-headed Gull (*Larus ridibundus*). Over the country generally, the Plover (or Lapwing) is one of the most beneficial birds to the farmer—in spring, its habitation is usually ploughed land. It undoubtedly destroys many injurious insects.

The Rook is not quite so wholly depraved as it is thought; for though it certainly takes a fair amount of seed corn, yet wireworms form about 9 per cent. of its food (Leigh(10)), and the young are fed on Elaterid larvae (Newstead, *loc. cit.*). Jackdaws and Starlings, both as nestlings and adults, feed on both larvae and imagines of Elateridae.

Curtis(6) states that the Sparrow preys on wireworms, but, up to the present, there is apparently no evidence available that might mitigate the uselessness of this ever-present pest. The author has little definite information regarding the particular proportion of the food of birds formed by the larva of *Agriotes obscurus*.

Wireworms appear to form a fairly constant constituent of the food of the common Mole (*Talpa europaea*), in fact insect larvae and worms form the greater portion of its diet. In an investigation on the food of moles(19) it was found that out of 100 moles, 41 of the stomachs contained wireworms. Though this investigation only extended over a short period (from Dec. 5, 1913, to Feb. 5, 1914), it yet serves to indicate

the value of moles from the economic point of view. Digestion in the stomach of the mole is apparently very rapid, thus rendering identification of the stomach contents difficult. It is, at any rate, certain that the animal is a very voracious feeder as Scheffer(15) found that *Scalops aquaticus*, a mole distributed over the eastern region of the United States, will consume more than its own weight of suitable food in a day. In the stomachs of nine moles which I examined it was found that three stomachs contained remains of Elaterid larvae (the remainder of the food was mainly various insect larvae, earthworms and slugs). It is interesting to note that in the districts where these nine moles were trapped, one farmer would not allow moles to be trapped, save when they were becoming too plentiful; on all the surrounding farms the moles were trapped regularly. The district has been examined for wireworms, and it was found that on the farm where the moles were protected, wireworms were very scarce, and then could only be found in small numbers in a very old pasture. On the surrounding farms, where moles were trapped, wireworms were taken in numbers. The animal is often a nuisance in grassland and cornfields, but it should be remembered that the mole is burrowing after food, and not for amusement. Again, as its food consists principally of insect larvae, and, as Adams(1) remarks, the mole does not hibernate, but continues active all the year round, it can well be seen how useful the animal is. These facts together with its voracity, and its need for meals at frequent intervals (Adams, *loc. cit.*), show the utility of the animal. It certainly destroys large numbers of wireworms, and should therefore be merely prevented from becoming too abundant. According to Graf(7), an important enemy of adult Elaterids is to be found in the Carabidae, species of *Calosoma* being particularly important in the control of *Limonius californicus* (sugar beet wireworm). Curtis (*loc. cit.*) mentions that the Carabs *Pterostichus madidus* and *Nebria brevicollis* are enemies of the wireworm. While collecting between 1914 and 1915, Carabid larvae and small adults (*Nebria*, sp.) were commonly found in potato drills, where wireworms were attacking the tubers. The adult Carabs most commonly found were *Pterostichus madidus* and *Nebria brevicollis*. It was found, as the result of carrying away two wireworms and a *Nebria brevicollis* in a tin box containing earth, that the Carab attacked the wireworms. On examination, after the day's work, the *Nebria* was alive, and the two wireworms dead and mutilated. Under natural conditions, no Carabs were seen attacking wireworms, though injured larvae were seen.

Wireworms with their tough highly chitinised skins, and underground habits, are almost free from internal parasites. Curtis(6), however, says that wireworms have a Hymenopterous parasite and quotes Bierkander as having found them. Forbes (*18th Rept. State Ent.* p. 47, 1891—2) records a parasitic fly as having been reared from a wireworm. Out of over 300 larvae collected, not one attacked by an internal parasite has been seen. Graf found the same result with 10,000 larvae of *Limonius californicus*. In this connection, I am informed by Mr Fryer that he has not infrequently found wireworms parasitised by what is probably a Proctotripid, at all events a Hymenopteron, but has never succeeded in breeding it out. No bacterial or fungoid disease was seen in any of the larvae in the field, or in the laboratory. In this connection, Graf reports as having occasionally observed fungoid attack in the field, but says that one fungus and two bacterial diseases were common under laboratory conditions. He also says that one bacterial disease was very fatal to the young wireworms, while the older stages seemed to be immune.

According to Leaflet number 10 of the Board of Agriculture (1916), wireworms are parasitised by a species of the fungus *Isaria*.

The pupae are difficult to rear; any disturbance or any slight variation in humidity or temperature, was sufficient to cause them to perish, and from the same causes some pupae passed into the perfect state but failed to mature. No disease of any kind was noticed. Graf notes that a few pupae of *Limonius californicus* were killed by a fungus.

Graf records a fungoid disease as having caused the death of adult Elaters under natural conditions.

Wireworms seem to be little affected by physical agencies under experimental conditions. They were found to be little affected by excess of moisture, but they could not resist dryness and were killed by frost. Under natural conditions it is probable that these factors are of no importance, as the larvae can always burrow down deeper in the soil, out of the way of frost, or in the search of greater humidity.

8. Summary of General Conclusions.

1. This paper contains an account of the larval and pupal stages of the Elater, *Agriotes obscurus*, whose larvae, together with certain other related larvae, are known as "wireworms."

2. The common "wireworm" in Cheshire, North Staffordshire, and South Lancashire, is the larva of *Agriotes obscurus*.

3. The life of the larva is probably four, rather than five years.

4. The larva of *Agriotes obscurus* can be distinguished from that of *A. lineatus* by means of the development of the blunt tooth, just below the apex of the mandibles (more prominent in *A. obscurus*); by the orderly arrangement of the body hairs, and the more anterior position of the stigmata; and by the pseudopodium or anal papilla on the ninth abdominal segment of *A. lineatus* having three apical divisions, while that of *A. obscurus* only has two.

5. Birds, particularly the Plover (or Lapwing), are of great value in checking the larvae of *Agriotes obscurus* (as well as other related species). The Plover, which is wholly beneficial, should be stringently protected. The common Mole (*Talpa europaea*) is also of great value in checking the pests, and should not be wantonly destroyed, unless increasing in too large numbers. The amount of damage caused by a mole is probably very small in comparison with the amount of good it does.

6. The larva pupates in an earthen cell in the ground, down to one foot deep; the pupal period is about three weeks; the imago remains resting motionless in the pupal cell for roughly two months, after which it comes to the surface, and hibernates under stones, clods, etc., until the next season.

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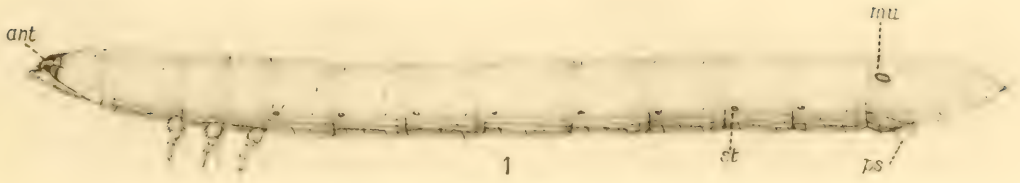

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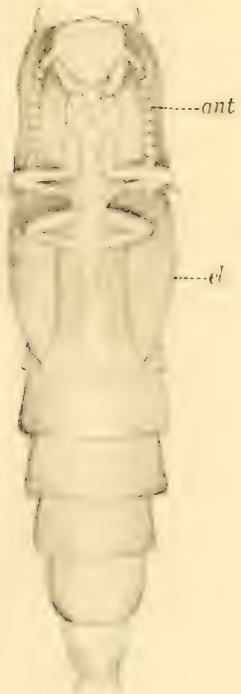

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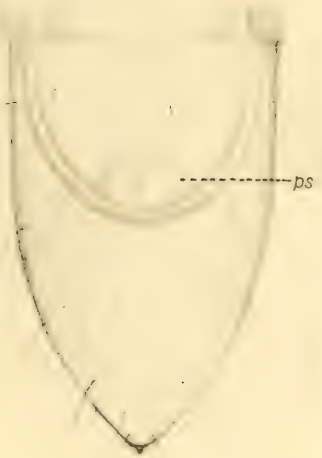

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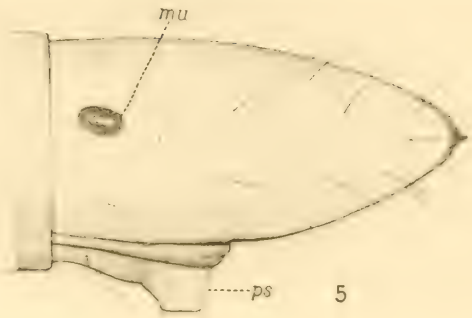

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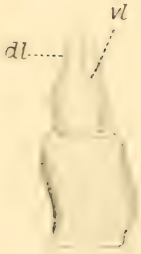

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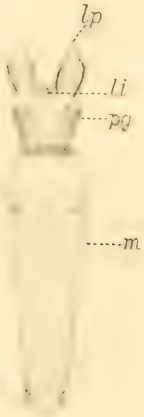

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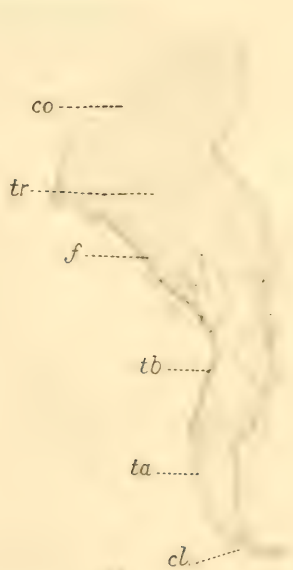

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DESCRIPTION OF PLATES.

PLATE XVI

FIGURE

1. Fully grown larva of *Agriotes obscurus*, lateral aspect, drawn from an extended spirit specimen. *ant.* antenna; *mu.* muscle attachment; *st.* spiracle; *ps.* pseudopodium. ×8.
2. Mature larva, as above, ventral aspect. *ps.* pseudopodium; *st.* first spiracle. ×8.
3. Pupa of *Agriotes obscurus*, ventral aspect. *ant.* antenna (left); *le.* elytron (left). ×8.
4. Pupa of same, dorsal aspect. ×8.

PLATE XVII

5. Lateral aspect of the ninth abdominal segment of the larva of *Agriotes obscurus*. *ps.* pseudopodium; *mu.* muscle attachment. × 24.
6. Ventral aspect of the ninth abdominal segment of the same. *ps.* pseudopodium. ×24.
7. Right mandible of the larva viewed from above. *gl.* ginglymus; *co.* condyle; *la.* lacinia mobilis. ×50.
8. Right first maxilla of the same viewed from below. *ca.* cardo; *st.* stipes; *mp.* maxillary palp; *la.* lacinia; *ga.* galea. ×50.
9. Labium of same, viewed from below. *m.* mentum; *pg.* palpiger; *lp.* labial palp; *li.* ligula. ×50.
10. Right antenna, seen laterally. *dl.* dorsal lobe; *vl.* ventral lobe. ×100.
11. Right leg of third pair of the larva. *co.* coxa; *tc.* trochanter; *f.* femur; *tb.* tibia; *ta.* tarsus; *cl.* claw. ×49.

ON THE BIOLOGY AND ECONOMIC SIGNIFICANCE OF *TIPULA PALUDOSA*.

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Part II. Hatching, Growth and Habits of the Larva[1].

(With Plates XVIII—XX and 3 Text-figures.)

The most common species of Crane-fly larva occurring in grass and corn land in the north-east of Scotland is *Tipula paludosa*. *Tipula oleracea* occurs also, but is much less frequently met with. Along with these, there has also been found in comparatively small numbers in fields the larval stage of *Pachyrhina histrio*, but this species appears to occur more frequently in garden ground. The following Tipulidae in addition have been found in the winged stage in the district surrounding Aberdeen:

Tipula varipennis, common and generally distributed.
T. gigantea, in small numbers.
T. lutescens, in small numbers.
Pedicia rivosa, L. widely distributed in the northern area, but not common.

The Egg.

Hatching of the flies goes on during the months of June, July, August and September, and as already recorded (Part I) the first mating and oviposition may take place within a very short period. In captivity, hatching mating and oviposition have all occurred within a few hours. A newly hatched female contains considerably over 400 shelled ova. In two such taken at random the actual numbers were found to be 448 and 490. A third female captured out of doors *in coitu* contained 255 black shelled ova together with a quite small number—about 12—of pale coloured immature shelled examples. The form of

[1] The work recorded in this series of papers has been carried out with the aid of Grants from the Board of Agriculture for Scotland.

this female when found indicated that oviposition had previously taken place. A female *Pachyrhina histrio* taken in the open contained 259 black shelled ova.

The egg measures 1·1 mm. × ·4 mm.; it is black in colour, with a dark purplish metallic lustre. As development proceeds this lustre diminishes, and finally before hatching the shell is of a dull black colour. The covering of the egg is a strong tough membrane, which is completely formed around the egg before copulation takes place. I have been unable to detect a micropyle, but this may be present. It is possible that the membrane before coming in contact with the air is permeable to the spermatozoon. The somewhat remote possibility of parthenogenesis taking place with the first brood of eggs has not been overlooked, and females have been kept apart from males from the period of their hatching until death, but oviposition never took place under these conditions.

The Early Larva.

The emergence of the larvae takes place in about 14 days after the eggs have been laid. They are then of a pale reddish sandy colour, and about 2·7 mm. in length, Plate XIX (*b*). When fully extended, thirteen body segments can be made out. On each of these from the third to the twelfth there is a small tuft of laterally placed, moderately strong bristles. The thirteenth segment which bears the spiracles and terminal papillae, has a pair of tufts of relatively stronger and longer curved bristles, borne alongside the large lateral conical para-anal papillae. These tufts constitute the most striking difference between the early larva and the later form.

Through the skin the two longitudinal tracheal trunks are visible, and also the alimentary canal with its four anteriorly and one posteriorly placed diverticula. The masticatory apparatus is well developed, both mandibles and first maxillae being strongly toothed.

In a short time the segmental bristles tend to become very short or worn away, and so also do the anal tufts. Traces of the lateral bristles persist even in the fully formed "grub," but the anal tufts disappear completely. These changes appear to come about by attrition. In about 12 to 13 days from the time of hatching the larvae are 4—5 mm. in length when fully extended, and already resemble the older and more familiar "leather jacket." By about three weeks they have attained a size of 6 mm. They feed from the first day onward.

The fully grown Larva: External features.

Owing to the difficulty of keeping alive recently emerged larvae which were prevented from burrowing into the soil, it has not been possible so far to follow the changes in external form effected at the various moults. The larva when fully grown attains at full extension a length of about 40 mm. It is now of a brownish-grey earthy colour interspersed with irregularly placed blackish dots. Frequently the longitudinal tracheal trunks, two in number, may be seen through the skin. The shape is cylindrical, slightly narrowed anteriorly, and expanded posteriorly into a peristigmatic papillae-bearing area. The skin, which is generally tense in healthy larvae, exhibits the following characteristics:—along each side there is a moderately wide strip which on the animal contracting folds outward, forming a pair of blunt keel-like longitudinal ridges. Besides numerous transverse wrinklings, there are slight but definite transverse furrows marking off distinct segments. Eleven of these can generally be counted. Each segment bears on its ventral surface four very minute bristles, two lateral and two near the middle line slightly in front of the lateral pair. On the first four segments behind the head dorsally, there is a row of bristles, and a pair of dorso-lateral bristles on succeeding segments. The head bears a pair of short jointed antennae; there is a very strong chitinous and highly complex mouth armature which includes massive toothed mandibles with palps, a pair of serrated first maxillae, notched united second maxillae and elaborately folded and bristled labrum (Plate XIX (*a*)). The whole set of structures is based upon a large strong bivalved chitinous support which surrounds the gullet.

The anus, which is sub-terminal, is surrounded by large fleshy lobes and a pair of large retractile laterally placed conical papillae. Beling regards these papillae as characteristic for this species.

On the somewhat truncated terminal region there is a pair of large brown coloured circular stigmata, each with a lighter glistening dull golden marginal ring. This stigmatic area is expanded on its border into six conical papillae, of definite form and arrangement. There is a ventral pair whose tips are black with a clear central area. This pair appears to have a sensory function, and may be seen at times in the living animal apposed to the stigmata above. Below each of these ventral papillae there is a pair of small pigmented spots which are sometimes united to form a short streak on each side. The remaining

four papillae project dorsally in two pairs. These bear on their exposed surface numerous fine hairs which follow the boundary of an elongated slightly raised conical area; the outer pair is tipped in black. Fig. 1.

Fig. 1. Stigmatic area in *Tipula paludosa*, (*a*) showing papillae with hairs, stigmata, and pigment spots; (*b*) showing anus and fleshy lobes, together with para-anal papillæ.

DP. Dorsal papillæ; LP. Lateral papillæ;
VP. Ventral papillae; AP. Para-anal papillæ, outside stigmatic area; A. Anus.

Duration of Larval Period.

In the N.E. of Scotland the adult flies may be seen frequenting cultivated land from early in June to the beginning of October. It appears to be the accepted opinion in England (Theobald, *Agricultural Zoology*, 1913, p. 228) that there are two generations of these flies, *T. paludosa*, and *T. oleracea*, in the course of the year. Our observations have shown that in this area, probably owing to the higher latitude and more rigorous climate, there is only one.

The following observations made upon a small collection of flies reared from eggs which hatched in September, 1913, are typical of the results obtained in rearing during several years. The parent flies had hatched out within small laboratory cages or had been caught upon the college farm and placed in these. The cages had wooden roof and

base, wire gauze sides and glass front. They contained a bed of turf two to three inches deep. Mating and oviposition readily took place, and on the turf being broken up later a considerable number of recently hatched larvae was found. A later search in the month of October, however, showed the mortality to have been considerable. On 1st November, the turf was again broken up and the surviving grubs collected and measured. The lengths were taken by allowing the larvae to crawl upon a sheet of paper and pricking this at the moment of their maximum extension. About one half the number of larvae was found to be under 16 mm. in length, and the remainder from 30 to 35 mm. It is remarkable that the larvae may attain to nearly their maximum length quite early, but it must be noted that they are relatively slender at this period. Subsequent growth takes place in the direction of thickness. By the end of the month the disparity in size was not so great. They all conformed to Beling's description of *Tipula paludosa* larva. In November clover was sown in the cages; this for a time afforded opportunities for feeding but was allowed to die out during winter. These larvae lived throughout the greater part of the following summer in soil containing decaying vegetable matter only, and duly pupated and emerged as adults in July and the early days of August.

As a control upon the above, portions of second year's grass upon the farm were dug up in October, the turf was disintegrated, shaken up in sieves, and the soil searched. Larvae were found and measured in a similar manner, and the commonest size at this date was found to range from 20 to 25 mm. These also were identified as *T. paludosa*.

Larvae collected out of doors during November and the first half of December showed a maximum size of 20 mm. These were relatively slender at this degree of extension, and contracted when handled to much smaller bulk than the laboratory reared specimens.

The larvae collected out of doors on 1st November, and which were separately caged, but kept under similar conditions, when fully extended measured 36 mm. in December, but in this state were distinctly more slender in the body. Examination of the contents of the alimentary canal showed that they had fed upon the decaying rootlets in the soil. As the season progressed it was found that there was eventually no significant difference in size between those reared indoors and those living a natural life outside. The records of soil temperature taken on the farm showed that there had been little frost during this experiment. The winter was a mild one, and the facts suggest that some feeding at least had taken place amongst the larvae out of doors. In a subsequent

season caged larvae kept short of food were found in February to measure from 20 to 30 mm. Generally, there has been found out of doors great variation in the size of larvae at the end of winter in the same district and even upon the same field.

Tipula paludosa has been kept under direct observation throughout its whole life cycle, and owing to variations in the length of the larval stage pupation and consequently hatching of adults is spread over a considerable period, viz., in this district, June to September. (Rarely, I have found adults in the cages in May.) Under experimental conditions of limited food supplies larvae have been kept alive and been continuously under observation for fifteen months. The minimum duration of the larval period has been found to be about nine months—September to June. Before all the larvae of a season have pupated the next season's larvae may have appeared, so that there may be larvae present in the soil all the year round. There is a possibility that this fact may have led to the view that there are two generations of flies in the year. I have had under observation in breeding cages in the month of July, larvae, pupae, and adults of one generation, together with developing eggs and emerged larvae of the next generation—all alive at the same time; and in the variable climate of the region under observation such occurrences are not improbable in the field.

Bionomics of the Larva.

The newly emerged larvae are very susceptible to drought, and when kept in dry soil were found to die off quickly. Strong sunlight, even when the soil is moist, was also fatal in a short time. Artificially reared larvae require to be kept moist and sheltered from direct sunlight, otherwise the mortality in the early days is very great. Larvae which are hatched from eggs which have been placed upon the surface of the ground immediately burrow into the soil, avoiding the light. A large proportion of larvae reared from eggs in 1913 died in the course of the first eight weeks, especially towards the end of this period, notwithstanding all attempts to reproduce natural conditions, and only a comparatively limited number of flies have been reared from many thousands of eggs laid in the laboratory cages.

In view of the fact that very large numbers of eggs are laid, and of the probability that the adults only rarely approximate to these numbers, there must be a considerable mortality in the course of the life-history, due, of course, to various factors. Our experience suggests that the first

few weeks of life constitute a period in which the insect is particularly susceptible to the prevailing physical conditions. While difficulty was experienced in rearing large numbers of larvae from the egg to the adult stage, no such difficulty was met with in rearing flies under the same laboratory conditions from larvae collected in the field in late winter. It may be suggested for example that wet weather in the end of summer, and early autumn months, will favour the survival—apart from natural enemies—of greater numbers of larvae, and that conversely, prolonged drought will tend to kill off numbers of those hatched about this time. In this connection it may be worth while to quote the opinion held by some farmers in this area that a wet summer and autumn foreshadows a plentiful supply of crane-fly in the following year.

The Larva on Farm Lands.

Published references to the activities of the larva as far as I have been able to trace them deal exclusively with instances of serious or even excessive damage effected by these insects upon grass or corn crops. But in the course of the present investigation it has become clear that Tipula larvae are very commonly present upon farm lands, sometimes in considerable numbers, without their presence becoming apparent. Cases of excessive damage have also been experienced, but the following instance may be taken as an average experience under the conditions named. It is quoted in full because it illustrates a number of features related to the larval habits.

These observations were made in the spring and summer of 1913, upon the College farm at Craibstone. Search was made for the presence of Tipula larvae in the end of March and beginning of April. The weather was cold at the time, and the searches were not very fruitful. The Woodlands field (Fig. 3) which was in grass at this time was selected for enquiry, samples being dug up at a number of places, and the turf thoroughly examined, but no Tipula larvae were obtained. Grey slugs were particularly plentiful. This was on 3rd April. On the 19th, ploughing was in progress and the plough was followed, samples of the furrow were taken, disintegrated, and searched, but no larvae were found in this way. Further search by two observers resulted in four larvae being found. These were found under stones at the surface, on the part not touched by the plough. After the field had been sown and rolled it was again examined on the 29th, and larvae were now found to be very numerous under the turf clods upon the surface. In

the interval between the times of examination there had been a good deal of rain.

On the 3rd of May the field was visited at 6 a.m. The two previous days had been dry and sunny, but in the end of April there had been much wet. The morning was fine, and at 6 a.m. the sun's rays had reached the western end of the field only. The eastern end was still in the shadow of the trees.

A search was commenced at the eastern end where there was some frost in the ground. In about 45 minutes 94 larvae had been collected. In the southern hollow where the sun had now reached, 42 were obtained in about 10 minutes; on the crest of the field at the west end (in sun) 75 were found in 20 minutes, and on a low part (N.W. corner), in the sun, 15 in 15 minutes. Two collectors were at work. In all in about 1½ hours 226 larvae were obtained. In some cases from six to a dozen were found in a single piece of turf. The smallest number appeared to be present in the shade at the highest level of the field (E. end). The larvae were found mostly under the turf clods, and largely in the "mids"; sometimes they were lying on the soil below, and sometimes embedded in the turf with heads well buried amongst the roots. They were not seen distributed generally amongst the sown grain.

The field was again examined on the 10th of May. There had been continuous and heavy rain for several days previously, and the ground was very wet. The oats had "brairded" early in the week, but the wet weather had rendered rolling impossible. At the time of search rain was not falling, but there was a mist close down to the ground. Larvae were frequently found beneath the loose turfs upon the surface, and they were also to be seen crawling freely on the ground. Some trouble was taken to find them in the act of attacking the young crop, but with no success. The ground in a number of places was scraped with a toothed digger and the plants turned over. A few larvae were found in the ground in this situation, i.e., free from turf clods and amongst the soil in which the oats were growing, but none was seen actually attacking any part of the crop. The oats were scarcely an inch above ground. In many places no corn could be seen and here the ground was turned with the digger. A few larvae were found in this way, but it could not be said that they were more numerous than in other situations. It was found that mostly the seed in these places lay deeper and was growing all right.

During the following week the weather improved and there had been several warm and dry days. On the 17th the field was again

examined. It had been rolled during the previous day. The crop looked well, and there were no indications of Tipula attacks. Larvae were again found in the most usual situation, viz., below the turf clods; 40 were collected in a few minutes. Search amongst the growing oat plants resulted in only one larva being found; none was seen upon the surface, and none detected attacking the crop.

On the 21st another examination was made. The weather in the interval had been showery, but not very cold. The day was warm and there was some wind. The field was carefully searched, particularly for traces of larvae moving freely in the soil or actually attacking the crop. They were found in the usual places below or burrowing into loose "foggage" upon the surface. In a few cases they were found below flat stones at the surface. Only a very few were obtained by searching the open soil around the oat plants. In this search the soil was turned over with a digger and the oat plants uprooted. Sometimes the ground was scraped and stirred. The examination ought to have discovered larvae if they were present in the soil in proximity to the roots of the oat crop, and it is concluded they were absent in this situation at the time of search, viz., between 10 a.m. and 12 noon. The weather at the time was warm and showery, and larvae could always be found amongst the decaying turf. The crop at this date, notwithstanding the undoubted presence of Tipula larvae in large numbers, showed no bad effects. Up to the beginning of July, when the last search for larvae was made upon this field, there was no apparent effect of the presence of Tipula upon the crop. On this occasion the search was effected by cutting the crop over certain areas, and sifting the soil by spade and sieve. Larvae were obtained, but no pupae were seen.

It should be mentioned that in view of the presence of Tipula in appreciable numbers and the possibility of an attack ensuing, a plot experiment was early arranged upon the field with the object of testing the effect of rolling and of some common manurial substances. The experiment, though negative in its results as far as its original purpose is concerned, is given here because it confirms the conclusion that Tipula was not visibly damaging the crop. The field, which is surrounded by trees, was rich in humic matter. Below is given a diagram of its situation, and of the experimental plots together with the report of Mr W. Findlay, N.D.A., Superintendent of Field Experiments.

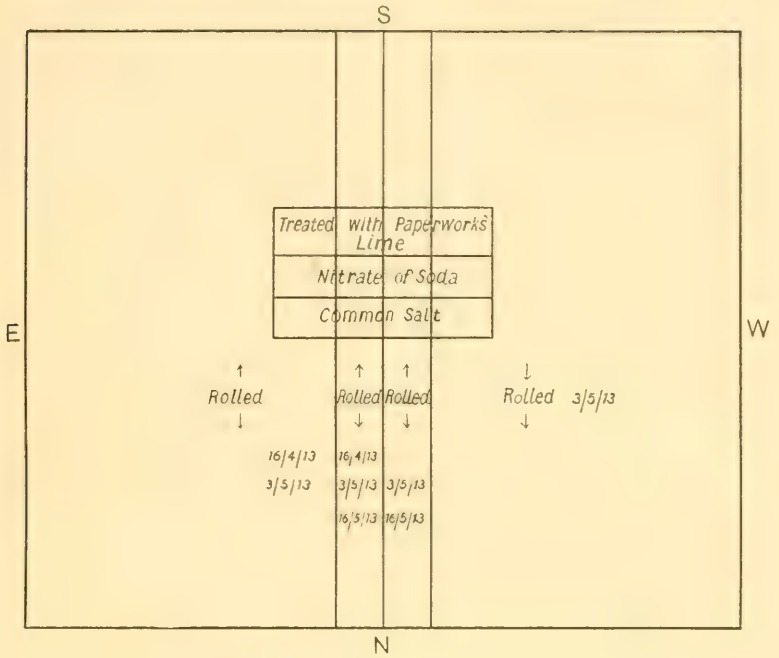

Fig. 2. Diagram of Experimental Area of Field.

Report. The Woodlands field at Craibstone was ploughed out of Lea during the second week of April, 1913, and immediately sown with Sandy oats at the rate of six bushels per acre. A heavy rainfall prevented the whole of the field being rolled at that time, and an interval of about two and a half weeks elapsed before the soil was dry enough to finish the operation. One strip the whole length of the field was rolled three times, and other two strips were rolled twice.

On the 21st May three plots were laid off and treated as follows:

1. 4 cwts. Paperworks' Lime per acre.
2. 1½ ,, Nitrate of Soda per acre.
3. 2 ,, Common Salt per acre.

The accompanying plan will show the scheme of the different treatments and cultivations.

At no time was there any difference either in the thickness or strength of the crop between the parts rolled at different times.

The plot to which Nitrate of Soda was applied showed an increased crop of about 20 per cent., but those to which Lime and Salt were applied could not be distinguished from the rest of the field.

WM. M. FINDLAY.

The experience here recorded has been general for a series of years; large numbers of Tipula larvae have been regularly obtained from lea fields upon farms on Deeside and elsewhere in the neighbourhood, which during the periods tested had no crop losses due to their attacks. In a good many such cases the numbers obtained from single fields were considerable. A case where serious damage was effected is given upon page 132.

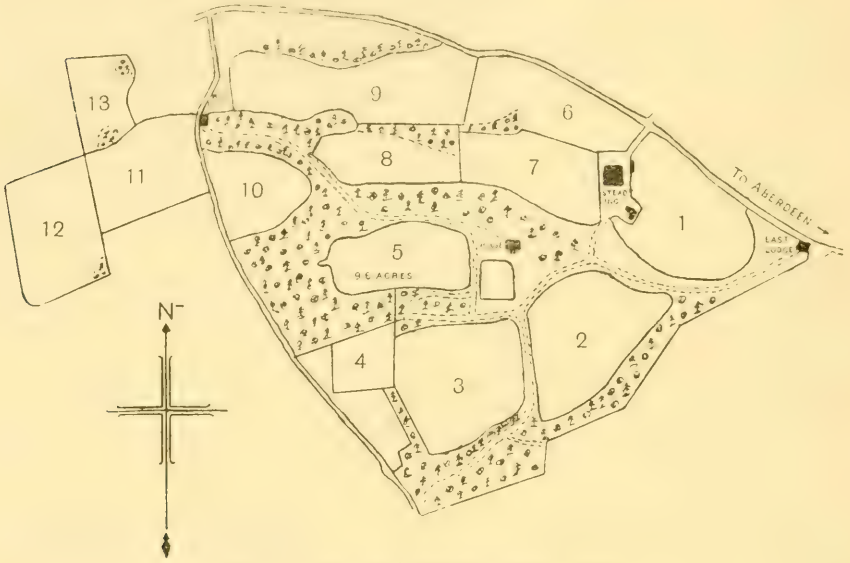

Fig. 3. Plan of Craibstone Home Farm.
Experimental Field, No. 5.

Summarising the outstanding features of this record we note: (1) An apparent scarcity of larvae in spring before ploughing took place. The failure to find Tipula was not as subsequent finds proved due to their smallness of size, and it does not seem likely that they were deeply situated in the soil at this time. They have rarely been found below six or eight inches under the surface. Tests made with deep cages in winter (Feb. and March) yielded only insignificant numbers (eight per cent.) below six inches from the surface.

(2) When known to be present in the field they could not be found in the act of attacking the crop. The suggestion that they feed at night is plausible, but observations on larvae in cages have shown that they feed readily at all times. The fact that there was an abundance of humic matter in the soil is probably not without significance in this connection (see pp. 127—8).

(3) The occurrence of most of the larvae at the surface beneath loosened decaying turf. Their presence here in the spring is general; they occur both loosely below, and also very frequently deeply embedded in the turf. The disturbance of the soil in ploughing and harrowing is probably the cause of their gathering in these situations, and their presence is probably primarily due to the need for shelter and moisture.

(4) The absence of harmful effect upon the crop notwithstanding their presence in considerable numbers throughout the spring and summer. This feature is considered in connection with the further data given below.

Further consideration of feeding habits.

With a view to rendering clearer the feeding habits of the larvae and to throw light upon the circumstances under which they attack growing crops numerous experiments were made of which the following are illustrations.

I. A small lot of larvae reared from eggs laid in September was kept in ordinary field soil covered with loose turf in a small laboratory cage. No crop was sown in the soil, but it was watered from time to time. They wintered under these circumstances and continued throughout the following summer. The larvae pupated in July and the last of them emerged as flies on the 4th August and mated on the same day. The life cycle in this case occupied about eleven months.

II. A collection of larvae was kept out of doors in small boxes containing ordinary garden soil without growing vegetation during the months of May, June, and July. They survived this treatment, but were undersized. Some managed to pupate, but others died in the larval stage. One imago was observed to fail in the act of emerging from the pupal case. The larvae of this group did not on the whole do so well as those of lot I, and the mortality towards the end of the experiment was high. Dissections showed the presence of vegetable fibres in the intestine, and a considerable amount of gritty material. It may be mentioned that this latter is normally present in the intestine.

III. A collection of larvae was kept in small cages with no growing plants, and a limited amount of decaying vegetable matter amongst the soil. These conditions were maintained during the months of May and June of the present year. At the end of June all were alive and healthy looking, and some were well grown. The cages were set in a large field rearing box containing washed sea sand, amongst

which the metal cages containing the larvae were sunk. A considerable number of larvae wandered from the cages and were subsequently recovered amongst the sand, alive and to all appearances quite healthy. Adult flies were later seen emerging from the cages in the laboratory to which they were removed during July. The larvae of this and Experiment II were collected in the fields and had wintered out of doors.

The foregoing, together with other experiments and observations of a like nature have shown clearly that the Tipula larva may subsist in the soil and complete its development independent of the presence or absence of a growing crop upon the ground. The results here obtained have led to the institution of further and more exact experiments dealing with the nutrition of soil larvae including Tipula. These are at present in progress.

IV. A number of larvae caged in the autumn of 1915 were kept in soil without growing vegetation except for a short period when a small quantity of corn sown in the cage germinated. In February they were found to have reached a fair size. Several killed and examined on the 19th were found to contain soil particles and fragments of vegetable fibre. At this date they were found mostly in compact earthen cells formed against the sides at the bottom of the cage. This habit has been frequently observed in winter and suggests a quiescent period under the adverse conditions of cold, confinement and restricted food supply.

In order to determine more clearly the circumstances inducing destructive attacks upon crops the following type of experiment was resorted to:

Groups of larvae were put up in large cylindrical glass cages of about 10 inches diameter, in prepared soil, in which the visible amount of vegetable matter was very slight. This soil was, further, mixed with well washed sea sand. Around the cylinders between the glass and the soil, oat seed was introduced. The cages were kept at room temperature and were examined daily. After the corn had germinated, the larvae were kept under close and continuous observation for prolonged periods at a time. The larvae appeared sluggish, and not much movement was seen in the day time, although their burrows soon became very numerous between the soil and the glass (Plate XX). They could be seen lying in these, and after the corn had germinated, or even before this, they could be seen attacking it, gnawing at the

husk, radicle and plumule. They were also seen eating the blades which had come above the ground. The glass cylinders had removable ends of perforated zinc of fine mesh. A few larvae passed through the perforations at the bottom although these were small Within the cage they tunnelled freely to a depth of six or seven inches. After a week, when all the corn appeared to have germinated, and both radicle and plumule were of some length, the cylinders were removed and the state of the seedlings ascertained. These were separated out carefully and placed in water. The soil was removed as far as possible by gentle washing, and each seedling examined in turn. Care had been taken that no other creatures were present in the soil capable of damaging the oats. There were usually about 25 larvae present. A typical result is given:

34 seedlings apparently sound.
11 had radicle or rootlets more or less eaten away.
39 had plumule cut through or bitten into. This includes a number attacked in the blade, above ground.
8 seeds were attacked. In some cases this had been effected before germination, in others, afterwards. In some of these the food store was completely eaten out, in others only partially so.

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Equal to 63 per cent. of the plants damaged.

The factors contributing to such a result appear to be:

(1) The absence of decaying or other vegetable matter, with consequent restriction to the crop for food supplies.

(2) The large proportion of larvae to the cubic content of soil—two dozen to about 1½ cubic feet of soil.

(3) The confinement of the larvae to a limited amount of space; it was not possible for them to leave the cages.

A further factor to be noted is the favourable conditions of warmth and suitable moisture, inducing rapid germination. This would be of some importance in the field in favour of the crop "growing away" in a proportion of cases before the damage done became irremediable.

In other experiments of this nature with germinating oats, clover, or timothy grass, the numbers of larvae used were very much greater, with correspondingly serious damage. It was found subsequently in examples of very severe attacks upon crops in the field, that the numbers used in these experiments were in excess, and that serious loss may ensue where the numbers, in average samples of an affected area, are about 10 to 15 per square foot of surface (see p. 133).

Migrations.

The use of large numbers of larvae upon experimental plots or open shallow cages brought to light the fact that scarcity of food in spring due to over stocking results in the larvae migrating. The first proof of this was obtained from a lot of 120 larvae contained in a small box in soil in which clover had germinated. As the food supply became exhausted the larvae left the box in large numbers and were found crawling about the laboratory. Two other instances of this occurred in connection with field plot experiments at Craibstone. In these experiments, small plots of $2\frac{1}{2}$ feet by 6 feet were walled off with wooden boards sunk six inches in the ground and standing about three inches above the surface of the soil. It had been established in experiments in a previous winter that these larvae even in very cold weather rarely went below this depth of six or seven inches, and it is not considered likely that they burrowed beneath the limiting boards of the plots. The plots were stocked with large numbers of larvae; in one instance three adjacent contained 900 larvae each and in other three plots, there were 700 each. When it was discovered that the crops in these had failed, at the end of June the soil in each was sifted for larvae. The hatching season had just commenced, but only a very few flies had been seen; of the 2100 larvae in the second group of three plots only 295 were recovered, together with a small number of pupae and two or three empty pupal skins.

Another plot was not disturbed. It had stood exposed in the same way as its neighbour, for some weeks, but later a large field cage was fitted upon it. Here there had been placed originally 2700 larvae, and now to these were added the 295 recovered from the other plot. In due course flies hatched out and appeared in the cage, but the numbers up to the end came very far short of those of the grubs introduced. The soil was searched and no trace in the form of empty pupal cases or dead larvae was found.

In a duplicate experiment on Holm Farm, in Lewis, carried out at the same time, designed to test the relative resisting or recovering power of selected oat varieties, it is probable that a similar migration took place. This experiment consisted of plots of Hamilton, Sandy and Potato oats, into each of which 900 larvae were placed. There was also a control plot of Hamilton oats, which received no larvae. The oats were sown on 18th May, and "brairded" on 3rd June.

The plots were under continuous observation till the end of June, during which time no difference was observed in the growth of the different varieties. At this date the plots were searched and only a few larvae could be found. The local observer states that he is confident birds did not interfere with the plots, and the only conclusion that can be drawn is that the larvae migrated from the plots before the crop had germinated. Had they remained and continued their development, pupae and empty pupal cases would have been found in the plots. As a matter of fact these were examined before adult flies had begun to appear. Had the larvae died within the plots it is certain that the remains of some at least would have been found, when the soil was passed through the sieve. But nothing of the kind was found.

The question remains—do they migrate in appreciable numbers normally, in search of food? On the fields under observation I have found them wandering upon the surface, but so far only occasionally. With regard to the plots, it is to be borne in mind that the larvae had been placed in excessive numbers on soil which did not contain any growing plants (except the ungerminated corn), while around there was abundant green vegetation. Their power to climb up even a few inches of a vertical board was not expected; that they did so was also evidenced by their being found in the control plots in which none had been placed.

A further interesting case, bearing on the point, is recorded in an editorial note in the *Scottish Naturalist*, 1915, p. 1.

The reference is brief and may be quoted in full:

"A few weeks ago it was reported to us that the inhabitants of a certain district in Perthshire had been seriously alarmed by an invasion —not of Germans—but of an immense number of small worm-like creatures which crawled over the road near the houses in such numbers as to make walking decidedly unpleasant. Examples of the creatures were brought to us, and were recognised as 'leather jackets'—otherwise the larvae of *Tipula oleracea*, the commonest species of crane fly."

As bearing further on this subject, the Editorial note proceeds to quote from an article by P. Desol, in *Compt. Rend. Soc. Biol.*, Paris, LXXVII. No. 21, June, 1914, pp. 126—7.

The summary given is quoted from the October 1914 issue of the *Review of Applied Entomology* (Series A). As the conclusions here are at variance with my own on *T. paludosa* and with the occurrences just described they are reproduced. The observations were made in the meadows of Avesnois. "In the spring of 1914, circular patches of from 15

to 60 feet in diameter, or the entire areas of meadows, were to be seen covered with yellow and dead grass the roots of which were found to be full of the earth-coloured larvae of *Tipula oleracea*. Grasses and clovers are chiefly attacked while plants with hard or thick roots are not affected. Where the infested zone borders on a furrow the larvae fall into it during the night and being incapable of climbing out may be collected in large numbers. They are not migratory, so that healthy meadows may be found close to infected ones. Infection is due to chance circumstances bringing fertilised Tipulids to the ground." I am satisfied that the statement that the larvae are not migratory is erroneous and that the existence of healthy meadows near them may easily be otherwise explained.

The conclusion to the experiments just described—unsatisfactory as regards the object for which they were planned—was due to the excessive numbers of larvae used on the plots. The large numbers were decided on with a view to avoiding an indifferent result, and this was supported by field experience, where large numbers had been seen and which had not proved harmful. In its result, however, it is useful and the possibility of migrations from waysides, waste land, or grass fields to germinated corn crops cannot be neglected. The experience recorded on p. 122 may be recalled, in which a field examined in April, and found almost negative as regards the presence of Tipula larvae, was later found to have an abundant supply. This problem is under continued investigation.

Various field experiments and observations have been made with the view of testing the effect of manurial agents and various insecticides upon the larvae. One of these is quoted below, but in general the results obtained were not consistent, and in cases of some well-known insecticides were not corroborated by direct tests upon larvae in confinement. The experiments were useful mainly in bringing into prominence some factors which are contributing elements affecting the degree of attack upon the crop, and which are considered in the succeeding section.

Field Experiment: Holm, Stornoway. Oat Crop.

In the previous season the field was grazed by cattle right on to the end of November, and with sheep and cattle on to January. Ploughing began early in January, and was finished by the end of February.

Weather.

From the beginning of October to the middle of March it was exceptionally wet and boisterous. There was practically no frost and the temperature was normally mild. The Farm is worked on the five shift system; the present tenant came to the farm in 1910, and previous to this the farm had been much neglected, especially as regards drainage and weeds. Oats were sown in the last days of March. After sowing, the field was immediately harrowed—one single and two double. It was not rolled till 23rd May.

The oats "brairded" in about a fortnight from sowing time and looked strong and healthy for a week. About 20th April the crop began to look sickly in parts and by the end of another week the presence and activities of Tipula larvae became apparent. The weather for some weeks after sowing was warm and sunny.

The state of the crop was reported to the College authorities in May, and on 24th May arrangements were made to treat the field experimentally. At this time, it showed parts fair, parts very thin, and fairly large tracts were quite blank. All over the field leather jackets could be readily found, and on the bare parts they were present in very large numbers; scores could be found near the surface in a square foot of soil. A part of the field including the worst portions was plotted as under:

Rolled twice / Rolled once		Plot No. 5	Plot No. 4	Plot No. 3	Plot No. 2	Plot No. 1
	Unrolled	No manure	4 cwts. common salt	1 cwt. Nitrate of Soda	2 cwts. salt	1 cwt. Nitrate of Soda

The manures were applied on the 6th of June, and two-thirds of the total area rolled early the following morning. On the 14th June one half of that part was rolled a second time. The other third was not rolled.

On June 26th the larvae were still to be seen, and active. Ten trial counts were made on square foot samples taken at random. These were dug to a depth of six inches and the larvae counted. The following are the numbers found: 4, 5, 4, 4, 5, 4, 12, 14, 14, 13. No sample

tested was found blank. The last four lots were from Plot 5, unrolled part. Plots 1, 3, and 4, showed improvement on the crop at this date.

On July 14th the results showed as follows:

Plot 1. Originally the thinnest. Recovery good.
Plot 2. Originally 2nd thinnest. Now same as Plot 1.
Plot 3. Originally the best. Much ahead of all.
Plot 4. Originally only medium. Second best.
Plot 5. Originally 2nd best. Worst of all.

There was no very obvious difference between the once and twice rolled parts of the plots, but the unrolled parts were decidedly poorer. Larvae were still active in the field at this date.

On 15th September the field was visited and the crop as a whole found to be in a very good condition, it having tillered remarkably well. The variety of oat was Hamilton. Signs of the larvae having been present were scarcely apparent, any patching being extremely slight. The nitrate plots did extremely well, the salt ones also were very good. From the appearance of the crop in May, it would have been impossible to have foreseen such an excellent recovery. Local circumstances did not allow of the estimation of the yield of the separate plots by weight per plot.

Factors favouring the Larva.

It may be regarded as well established that Tipula larvae are generally distributed and ordinarily present in the soil, though not necessarily only upon farm lands. Even upon these they have been found to be present in appreciable numbers, when no recognisable loss in crops resulted. The question of importance is,—what are the circumstances determining a destructive attack upon a crop?

Naturally the first condition of importance is the presence of excessive numbers. The various factors contributing to the periodical appearance of large numbers of any species of insect have not hitherto been appreciated in advance, and indeed cannot be said to be well understood. In general with regard to Tipula they may be held to include:

(1) favourable weather conditions for the survival of the large numbers of young which are generally produced:
(2) unfavourable conditions for competitors:
(3) ready supply of food:
(4) absence or diminution of numbers of natural enemies.

As regards the influence of the weather in affecting numbers it has been already noted that the newly hatched larvae are very susceptible to drought and that the mortality in laboratory reared larvae which are not kept moist is high. There is a fairly general popular impression that severe winter with plenty of frost kills the larvae, but our experience so far does not bear this out. Larvae were left in the open exposed upon stones during a night of severe frost, and were found alive the following morning. The apparent beneficial result following frosts seems to be due to the improved tilth favouring a more rapid primary growth of the crop in spring. The environmental factors are the subject of continued investigation. Unfortunately, owing to the presence of the larvae on cultivated land, the food supply is adequate. Food is not ordinarily scarce, and as already indicated a high percentage of larvae kept in captivity complete their development upon ordinary turf in the soil.

The question of natural enemies is dealt with in a succeeding paper.

Tipula attacks on Oats.

Conditions unfavourable to the oat crop may render it susceptible, and Tipula, even when the numbers are not excessive, may work havoc. Apart from the question of manuring and general farm practice which are not considered here, the weather in spring has been found to be a significant factor. These observations have been carried on during a period of seven years; along with these the experience of over 130 farmers on a wide area has been collated and there is a universal testimony to the fact that a cold late spring in which the primary growth of the plant is delayed constitutes one of the most certain conditions for crop failure due to Tipula attack. It is during the early days of growth from the time of sowing until the adventitious root system is established that Tipula attack results in the destruction of the individual plant. After this period the plant may be regarded as out of danger; it may be weakened but probably it will not be killed outright by subsequent attack. Any cause therefore tending to extend the period of germination or immediately subsequent growth increases the liability of the crop to loss from Tipula attack. In this area the time between sowing and brairding for oats in an average season is 10 to 14 days. In a series of seven cases which came under observation in one season in which failure of parts of the oat crop, attributed to Tipula attack, took place, this

period ranged from 16 to 21 days. There was one case in which, owing to severe drought, it lasted six weeks. The actual proportions of crop failure were stated thus:

Period between sowing and germination.	Proportion of crop lost
16 days	one-fourth of whole crop
6 weeks	one-third of whole crop
17 days	one-tenth of whole crop
3 weeks	loss incurred; proportion not estimated
16–18 days	almost one acre destroyed
3 weeks	one-third of crop
3 weeks	one-third of crop; it recovered later

On this account early sowing, especially in our north-eastern climate, is attended with a certain amount of risk. The same risk would apply to all late districts in seasons when fine weather early in spring tempts the farmer to sow early.

Tipula and Oat Varieties.

The question of the relative resisting or recovery power of different oat varieties in relation to Tipula attack has been investigated both in the field and in plot experiment. The experience of the farming community within the College area has been obtained and discussed, and the general view is given below. There exists some opinion that the larva exercises a selective capacity and prefers some varieties to others, as is evidenced by the following statements:

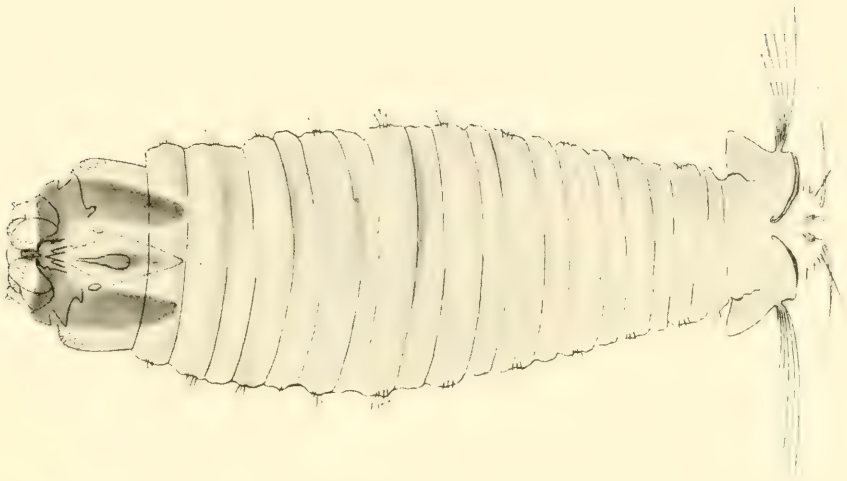
"They (i.e., the Tipula larvae) like Potato oats better than Sandy."
"Potato oats are more liable to attack than Red Oats or Sandwich."
"They are more fond of 'Potato' varieties."
"Waverley suffers from attack more than Potato or Sandy."
"'Banner' oats suffer more than Potato."

All the evidence, however, goes to show that this is not the correct interpretation. It is not the larvae which "prefer" particular varieties, but particular varieties which show better power of recovery from their attack. Some of the writers expressed their experiences more carefully:

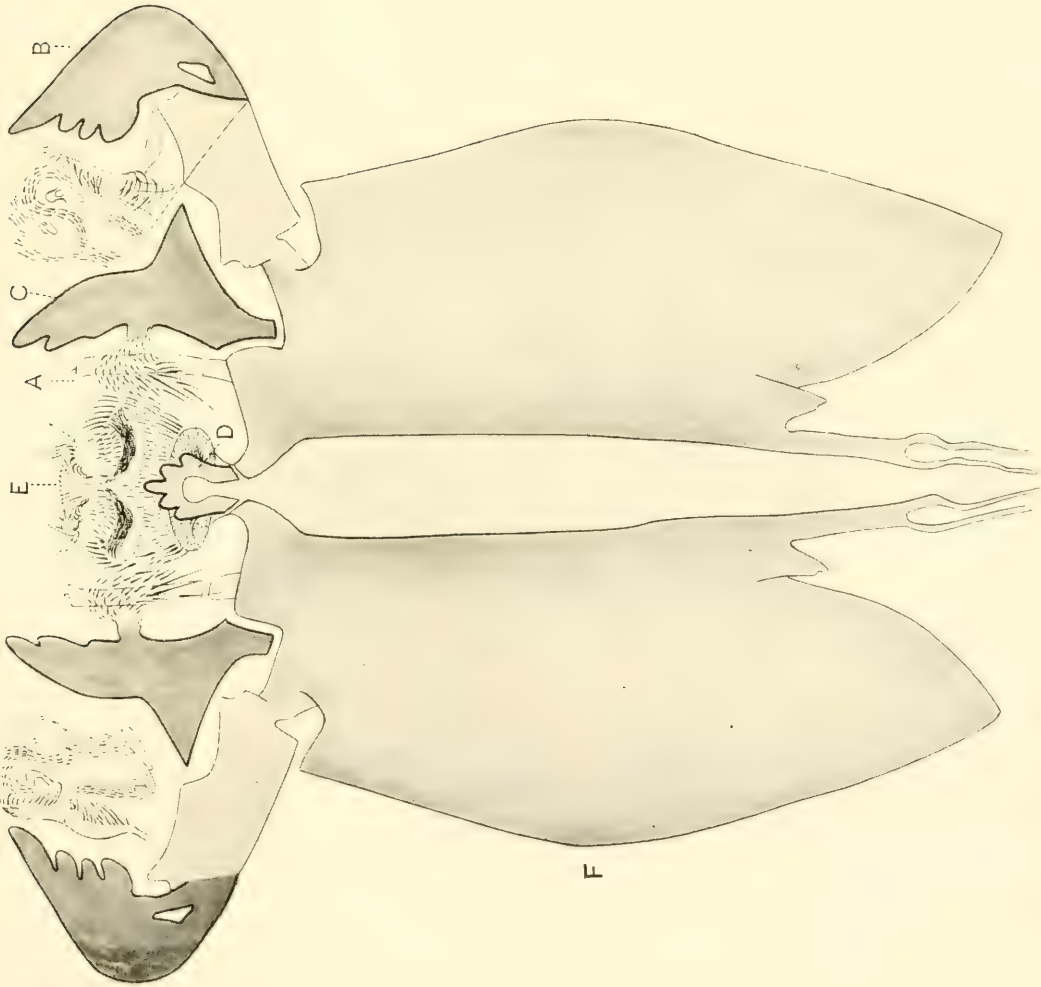
"Of the 'Waverley' and 'Hamilton' oats which were sown side by side, the 'Waverley' had to be resown; 'Hamilton' oats were only slightly thinned."

"The 'Red Oats' resisted poorly, 'Providence' and 'Potato' did better."

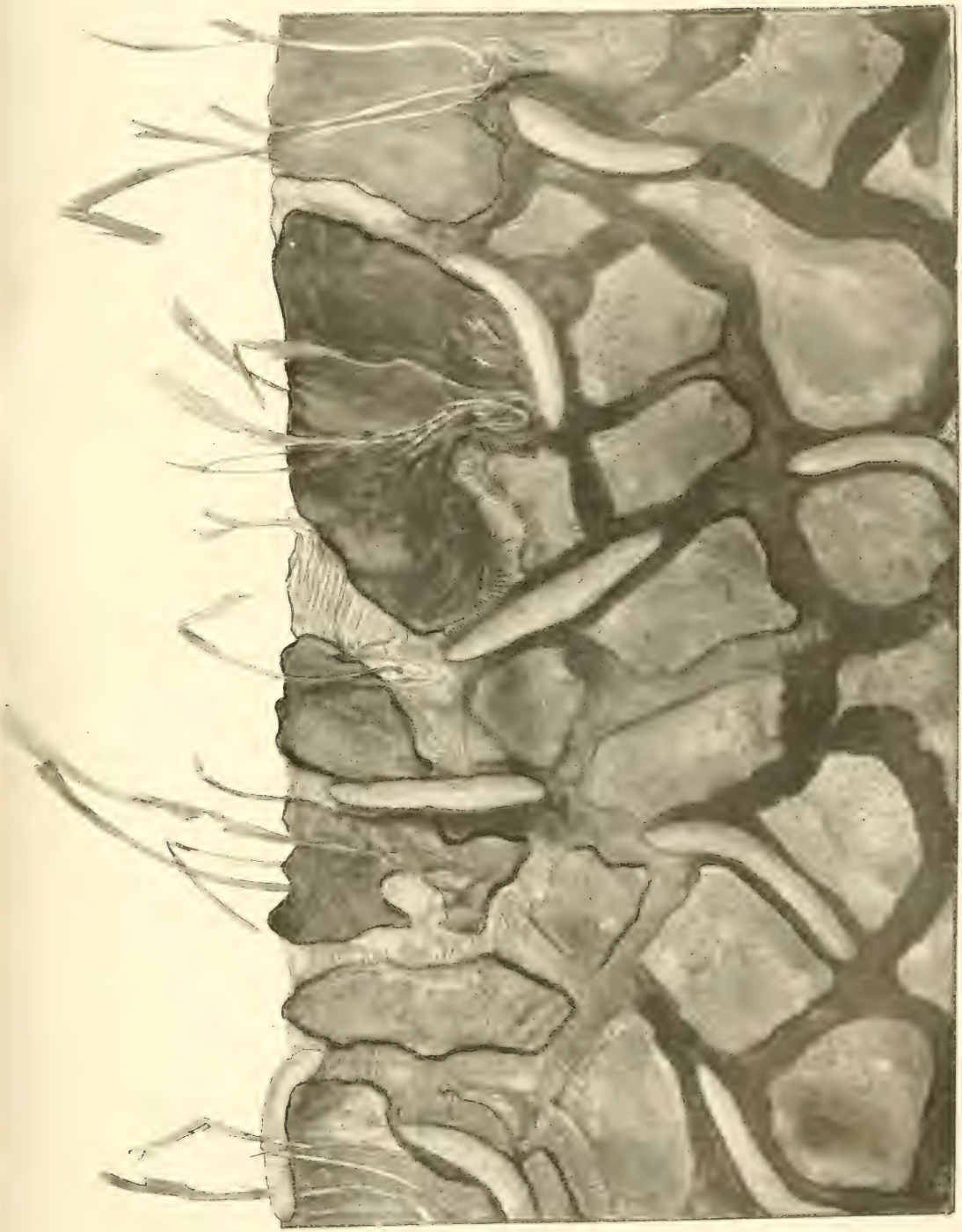
Taken collectively the views of over a hundred farmers who have had experience of Tipula attack is contradictory with regard to the



Del. E. Harvey (a)



Del. B. Simpson (b)



Del. B. Simpson

relative merits of particular varieties. This is likely to be the case so long as other factors such as quality and source of seed, type of soil, particular agricultural practice, manuring, and so on, are not considered. But there is general agreement that the newer varieties of oats have distinctly less power of recovery, because they do not tiller well. This is well borne out by wide experience.

Another practice which has been found of service in overcoming Tipula is to effect a "change of seed." Seed grown on the coast and sown in an inland locality, and seed grown in the Lothians and sown in Aberdeenshire have in both instances proved more resistant where larvae were at work, than native grown oats. Further, seed from an early district and sown in a late one, also from a light soil to a heavy one, have in both instances been proved satisfactory measures against losses from Tipula attack.

The subject of preventive agricultural practice and remedial measures in relation to Tipula are dealt with in a further paper.

REFERENCES TO PLATES.

Plate XVIII. *Tipula paludosa*. Larvae. 3 times natural size.

Plate XIX. *a*. Head armature of *Tipula paludosa*. (A) antenna; (B) mandibles; (C) first maxillae; (D) fused second maxillae; (E) labrum; (F) internal basal support of appendages.

b. Newly hatched larva of *Tipula paludosa*.

Plate XX. View of larvae in soil within glass rearing cage, showing their burrows and damaged oats, which were grown between the glass and the soil. Sketched from nature.

REFERENCE.

Beling, Th. Beitrag zur Naturgeschichte verschiedener Arten aus der Familie der Tipuliden. *Zool. Bot. Gesellsch. in Wien*, xxiii, Bd. 1873, pp. 575–592.

NOTE ON ATTACKS OF *PHYLLOTRETA VITTULA* ON SPRING CORN.

Phyllotreta vittula has recently been recorded by several continental writers as a serious pest of spring corn. It has also been recorded by Lind Rostrup and Ravn[1] as a serious pest of winter barley in Denmark. The following extract from Baranov's account of this pest in the Government of Moscow is taken from the *Review of Applied Entomology*, Series A, Vol. I, page 214. "This beetle becomes yearly more numerous and the damage done by it to the summer-sown crops is increasing. The author never found it or noticed any damage done by it on the winter-sown crops. It winters in the province of Moscow in the imago stage among the roots of summer stubble and of *Triticum repens*. It emerges from the earth with the return of warm weather (the middle of May in 1912) and immediately attacks the sprouting grain. It does the most injury at the time of pairing, and at the beginning of June its injurious activity decreases. It feeds on the epidermis and parenchyma of the leaves, forming channels in them parallel with the veins. If the number of channels is not great the leaves may recover. The author did not succeed in finding eggs of this insect. The harm done to the plants by the larvae was considerably greater than that done by the adults, and the author noticed that the former sometimes pass over to another plant. The plants attacked by the larvae generally die or are greatly retarded in their development. After the 15th June the insects decreased in numbers and by the beginning of July there remained only single individuals, but they reappeared on the 15th August in great numbers."

On April 27th, 1914, the writer received from Mr E. S. Beaven of Warminster, Wilts, specimens of seedling barley plants in which long strips were eaten between the veins of the leaves, very similar to the damage described above. With these plants were sent specimens of *Phyllotreta vittula* which were said to be present in large numbers and eating holes in the young barley plants soon after they come above the ground. The attack was not so severe as that described by Baranov.

[1] *Rev. App. Ent.*, Ser. A, Vol. III, p. 698.

This year a similar attack was noticed at the Rothamsted Experimental Station by Mr A. W. Rymer Roberts, who writes as follows: "On one of the fields at the Rothamsted Experimental Station I noticed during the course of last May considerable damage done by *Phyllotreta vittula* to young barley of three or four inches in height. The field had been ploughed out of ley the previous winter, and though not ordinarily one of the experimental fields, had this year crops of various kinds in strips. In addition to the barley, spring-sown oats on the next strip were also affected, though not so severely as the barley.

"The beetles usually seemed to attack the upper surface of the leaf first, eating through the parenchyma and leaving only the epidermis of the under surface. The holes so made, as you will see by the photograph I am sending, are in general oval, running between the ribs of the leaves, as Baranov describes; only in our case the pest was not bad enough to render the holes confluent, nor did I notice that plants were killed by it.

"The beetles disappeared about the end of the month, and I did not notice a second brood, though I may have overlooked them owing to pressure of other work.

"Though the damage done was not very material in permanent injury to the crop, in view of the seriousness of the attacks on spring-sown corn in Russia, it will be well to watch for any extension."

It will be noticed that the attack at Warminster was earlier than that at Rothamsted which occurred at about the same time as that of Baranov's. The attack in both cases occurred when the plants were very young, and therefore at a time when the plant is most likely to receive a severe check.

As this pest is so widely spread on the Continent, and has occurred at such widely separated places as Rothamsted and Warminster (where attacks would not easily be overlooked) it is possible that the damage done by this pest in other parts of England may have escaped notice.

F. R. PETHERBRIDGE.

School of Agriculture, Cambridge
December 1916.

ACCESSORY WETTING SUBSTANCES WITH SPECIAL REFERENCE TO PARAFFIN EMULSIONS.

BY A. H. LEES, M.A.,

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WITHIN the last three years or so the opinion has been gradually gaining ground that one of the most important points in a spray fluid whether for fungicidal or insecticidal purposes is its wetting power. The author's attention was first called to this point when using certain proprietory insecticides which, used according to the directions given, were not very effective but which, on addition of soft soap, had their wetting powers and therefore their killing powers greatly increased. Reference was made to this subject in the *Annual Report for* 1913 *of the Research Station, Long Ashton*[1], and since that time other papers have appeared dealing more fully with the physical problems involved in the question[2,3]. It is not proposed here to touch this side of the problem as the author is not a physicist but to give certain results bearing more on the immediately practical aspects of spraying.

It has long been known that the addition of soft soap to a fluid containing no substance capable of precipitating soap, causes an increase of wetting power, No quantity of soap, however, has proved sufficient to wet such resistant insects as the colonies of Woolly Aphis which occur on apple trees. These insects excrete a covering of waxy threads on their back which render them impervious to ordinary spray fluids. The same difficulty was found in the experiments of Prof. Barker and the author against American Gooseberry Mildew. There was therefore a real need to find a satisfactory accessory wetting agent.

[1] *The Annual Report of the Agric. and Hort. Research Sta., Long Ashton*, Bristol, p. 70.

[2] Lefroy, "Insecticides." *Ann. App. Biol.* Jan. 1915.

[3] W. F. Cooper and W. H. Nuttall, "The Theory of Wetting and the Determination of the Wetting Power of Dipping and Spraying Fluids containing a Soap Basis." *Journ. Agric. Sci.* Vol. VII, pt. 2, Sept. 1915.

In this connection paraffin emulsions were tried. These emulsions have been often recommended by many writers as insecticides and there is no doubt that they are of value. They have, however, a reputation of being dangerous to use owing to their liability to scorch foliage. There are, the author believes, very definite reasons for this liability but by suitable means it may be got rid of.

An emulsion may be stable or unstable, its state depending on its constitution. If stable, every small paraffin globule is surrounded by a film of soap and the film is sufficiently strong to keep one globule from touching and coalescing with its neighbour. If very little soap is originally present, or if where present in sufficient quantities it is withdrawn by chemical means, there comes a point where the film is no longer strong enough to prevent paraffin globules from touching and fusing with their neighbours. When this happens throughout the emulsion, paraffin appears in quantity on the surface and the emulsion is said to be unstable.

Pickering[1] has pointed out the great diversity of formulae for paraffin emulsions that have been recommended. In a table given by him the proportion of paraffin to soap in one formula is as great as 100 : 1·2, in others as low as 100 : 240, while there are all kinds of intermediates. This enormous range in quantities suggests that some of these formulae have been advocated in a somewhat haphazard spirit, since if formulae at one end of the series were successful it does not seem necessary to make use of others which contain such divergent quantities.

Of the 24 formulae quoted 15 contained paraffin in proportion to soap in ratios varying from 100 : 23 up to 100 : 1·2. That is to say in those mixtures of these fifteen where the paraffin bore the lowest ratio to the soap, it was present to the extent of four times the amount of soap and in those which bore the highest ratio, to as much as eighty-three times. It was therefore at first sight probable that the liability to scorching was caused by the high content of paraffin. Experiments were therefore started to see how little paraffin need be used in order to be lethal and at the same time to avoid scorching. It was at once found that with the Bristol water which has a hardness of about 12° it was impossible to obtain any kind of stable emulsion with even small quantities of paraffin unless at least $\frac{1}{2}$ % of soft soap were used. The paraffin simply floated on the surface of the liquid and when sprayed on to foliage naturally produced scorching.

In this connection it is interesting to notice that out of 23 formulae

[1] *6th Report, Woburn Exper. Fruit Farm*, 1906.

quoted by Pickering in which the amount of soap was stated, in 7 it is ·5 % or lower. In these 7 therefore, supposing hard water had been used, however little paraffin were added, scorching would be probable. In the others where the paraffin content was relatively high and the soap content relatively low there would also be a likelihood of scorching since, as will be shown later, the amount of paraffin that can be held in stable emulsion depends largely on the amount of soap present.

Preliminary experiments had shown that it is possible to obtain wetting from paraffin emulsions in two ways. If the ratio paraffin to soap was low, wetting, if it occurred, was due to the aqueous solution, while if the ratio was high, wetting was due to a paraffin film. It was clearly necessary to avoid this wetting by paraffin as to it, moderate scorching, when it occurred, was probably due.

In order to test the properties of paraffin emulsions of different formulae with regard to stability and wetting power a series of experiments was started in the laboratory. The percentages of paraffin tried were, 0, 1, 2, 5, 10, 15, 20 and 25 and the percentages of soap $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2. Mixtures were made with both distilled and tap water. Each emulsion contained 100 c.c. of water and was made by syringing the mixture of soap and paraffin, without the use of heat, several times through the "rose" of a garden syringe.

Two points were then investigated:

(*a*) The wetting power of the mixture,

(*b*) Its stability.

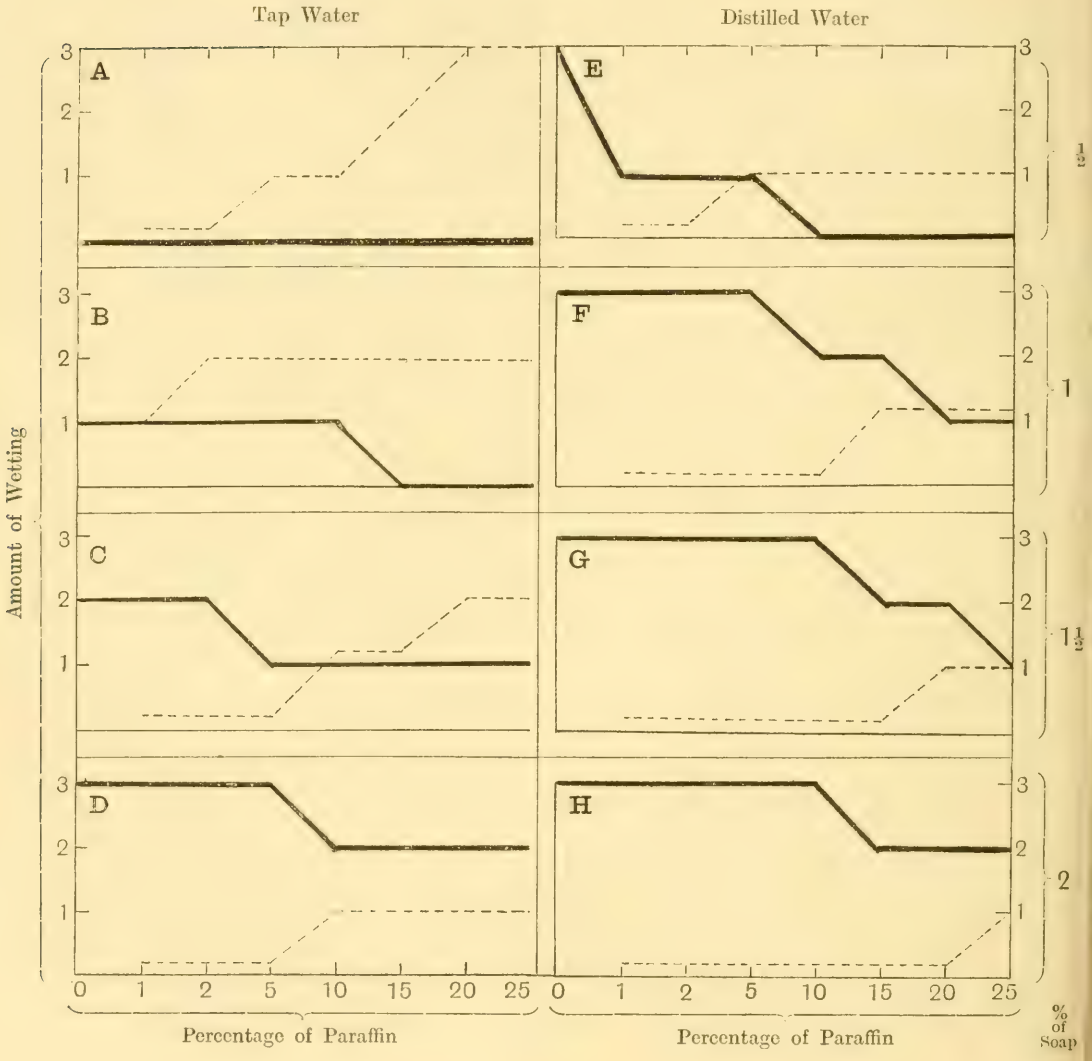
Wetting Power. As these experiments were done during the winter it was necessary to select some surface other than insects or fungi on which to test the wetting. The glossy American cloth cover of an exercise book proved a fairly good substitute. When the surface was worn by the action of the liquids used it was renewed by applying a thin film of boiled linseed oil which, when dry, gave a new brilliant surface.

Wetting took place either by means of the aqueous solution or by the paraffin or by a combination of both. A drop or two of the liquid to be tested was placed on the American cloth surface and gently made to roll. If the drop immediately spread and would form a thin stable aqueous film it was given 3 marks for aqueous wetting. If it spread but would not form an absolutely stable thin film it was given 2 marks, while if it wetted slightly only it was given 1 mark. If the drop failed to spread much and left the faintest film of paraffin behind it on

being made to roll it was given 1 mark for paraffin wetting, if the paraffin mark was definite, 2 marks and if abundant, 3 marks.

In the accompanying table the continuous line represents wetting by water and the dotted line wetting by paraffin. For the sake of clearness, where the dotted line lies along the zero line or along the thick line, it has been raised slightly above.

TABLE I



In considering these plotted results it is probable that as soon as the paraffin wetting rises above zero the mixture would have a slight tendency to scorch and a much greater tendency when wetting by paraffin attained a higher value than wetting by water. Considering the results obtained with tap water first it will be noticed that with only $\frac{1}{2}$ % of soap no wetting by water could be obtained and that with increase of paraffin the wetting by it increased to a maximum. The lower percentages therefore would be unsafe and the higher percentages would certainly produce scorching effects.

With 1 % soap there would be a slight tendency to burn even with only 1 % of paraffin but in practice, owing to the small quantity of paraffin present, this does not occur. With larger quantities such as 5 % and over burning does actually take place. With $1\frac{1}{2}$ % of soap the doubtful point is only reached with 10 % of paraffin and there would be no serious danger until 20 and 25 % was reached. For 2 % of soap probably all strengths would be safe, though for tender foliage there might be some small risk above 5 % of paraffin. With distilled water the doubtful points occur at 5 % for $\frac{1}{2}$ % soap, at 15 % for 1 % soap, at 20 % for $1\frac{1}{2}$ % soap and at 25 % for 2 % soap.

It will be noticed that the $\frac{1}{2}$ % distilled water results are intermediate between the 1 % and $1\frac{1}{2}$ % tap water results. This indicates that in water with a hardness of 12° (such as we have in Bristol) about $\frac{3}{4}$ % soap is used up in precipitating the metallic salts which cause hardness. That amount of soap is therefore wasted. (This represents about $\frac{3}{4}$ lb. per 10 gallons and involves a loss of about one penny for that amount of spray fluid.)

From a practical point of view the following recommendations may be drawn from these results. Using tap water of a hardness of 12° or using soft water the amounts of soap to be used for various percentages of paraffin in order to obtain a safe mixture are shown in the following table:—

Table II.

Tap water		Soft water	
% paraffin	% soap required	% paraffin	% soap required
10—25	2	20—25	2
2—10	$1\frac{1}{2}$	10—20	$1\frac{1}{2}$
1	1	5—10	1
—	—	1—5	$\frac{1}{2}$

In cases where the hardness of water is more or less than 12° the greater or less quantity of soap may be approximately calculated by assuming that 12° hardness equals $\frac{3}{4}$ % soft soap Thus for water of

24° hardness $2\frac{1}{4}$ % soap will be required for a 10 % paraffin emulsion while for only 6° hardness only $1\frac{1}{8}$ % will be required.

Stability of Emulsions. In considering stability of paraffin emulsions it must be pointed out that none can be kept indefinitely without de-emulsification setting in. For practical purposes it is sufficient to study their behaviour after comparatively short periods of time following their manufacture. If they will remain stable for one hour it is sufficient for practical purposes and experience shows that if any decided de-emulsification is going to set in it does so in the first hour. In the laboratory, therefore, after the various emulsions had been tested for wetting power they were poured, after thorough agitation, into burettes and allowed to stand for one hour.

It was found that all those formulae containing only $\frac{1}{2}$ % of soap in tap water were unstable and therefore dangerous to use. This agrees with what might be expected from the plotted results of wetting power, where the line indicating wetting by paraffin is always equal to or above the line indicating wetting by aqueous solution. Similar emulsions made with distilled water were stable even in cases (paraffin 10 %—25 %) where, in the plotted results, the paraffin wetting line is above the water wetting line. These emulsions therefore, though not certain to scorch, cannot necessarily be classed as safe. With 1 % soap and tap water the emulsions were still slightly unstable, as might be expected from the plotted results where the paraffin wetting line is nearly always above the water wetting line. These emulsions are therefore dangerous except in those cases where the paraffin content is low. Most plants will stand a very small quantity of free paraffin if well distributed, since, under conditions of spraying, much of it is evaporated before it can produce scorching effects. When however much is present scorching is bound to occur. The other emulsions tried, namely 1, $1\frac{1}{2}$ and 2 % with distilled water and $1\frac{1}{2}$ and 2 % with tap water, gave stable emulsions. It will be seen that in the case of $1\frac{1}{2}$ % soap and tap water at 20 and 25 % the paraffin wetting line was above the water wetting line, indicating that the emulsion was unstable. Actually however when tested for an hour in a burette no appreciable de-emulsified paraffin appeared. This should not be taken to indicate that it is a safe emulsion to use. It contains a high content of paraffin which is only just held in emulsion by the soap. In other words the soap pellicule that separates the paraffin globules is nearly as thin as it can be without actually parting asunder. So long as the liquid is at rest it is stable, but when forcibly beaten against a solid object as in spraying,

the soap pellicules break and liberate a certain amount of free paraffin. Such an emulsion is therefore unsafe to use. It is thus possible to divide emulsions into three classes:

1. Dangerous. 2. Unsafe. 3. Safe.

The first includes those which, like the $\frac{1}{2}$ % soap with tap water, de-emulsify on standing. The second, those like $1\frac{1}{2}$ % soap with tap water and 20 or 25 % paraffin, which de-emulsify on being sprayed. The third, those like the 2 % soap emulsions, in which no de-emulsification occurs at all.

Wetting Power of Paraffin Emulsion compared with other Auxiliary Wetting Agents.

In the previous portion of this paper the wetting power of paraffin emulsions on a certain artificial surface, American cloth, has been considered. Table III gives the results of the wetting power of some of these emulsions and of other auxiliary wetting agents on certain natural surfaces.

Table III. *Wetting Power of Various Fluids.*

Wetting Substance	Gooseberry Leaf	Sea-kale Leaf	American Gooseberry Mildew
Water	Nearly complete	None	None
Gelatine 1 in 1000	Complete	Very slight	,,
Gelatine 1 in 10,000	,,	,,	,,
Casein 1 in 1000	,,	,,	,,
Casein 1 in 10,000	,,	,,	,,
Soap $\frac{1}{2}$ %	,,	Slight	,,
Soap 1 %	,,	Nearly complete	Very slight
Soap 2 %	,,	,,	,,
Emulsion Soap 1 % Paraffin 1 %	,,	,,	Moderate
Emulsion Soap $1\frac{1}{2}$ % Paraffin $1\frac{1}{2}$ %	,,	Complete	Nearly complete
Emulsion Soap 2 % Paraffin 2 %	,,	,,	Complete
Emulsion Soap 1 % Paraffin 5 %	,,	Wets by paraffin but not by aqueous solution	Nearly complete
Emulsion Soap 2 % Paraffin 1 %	,,	Complete	Moderate

These surfaces were chosen to represent types that could be wetted with ease, with moderate difficulty and with great difficulty. They are a gooseberry leaf, a sea-kale leaf, and the white felted stage of American gooseberry mildew. The gooseberry leaf is smooth without being waxy and is easily wetted, the leaf of sea-kale is slightly waxy and is only wetted with moderate difficulty, while the surface of American gooseberry mildew offers great difficulty.

Of the other wetting agents soap, gelatine and casein were tested. Both the latter have recently been recommended, the former in an acid or neutral medium, the latter in an alkaline. For both one part in a thousand is relatively strong while one part in ten thousand is relatively weak; they were therefore tested at those strengths. Even at the weaker strength both increased the wetting power but the increase was only slight. They caused complete wetting of the gooseberry leaf but only raised that of the sea-kale leaf from none to very slight while the effect on the mildew was nil. For waxy and hairy surfaces, therefore, they are useless though they are to be recommended where extra spreading power is desired on an easily wetted surface. With arsenate of lead and more especially with Bordeaux mixture, where a cheap substance is required to obtain even spreading, they are most efficient.

Soap and paraffin emulsions having a soap basis may be considered together as they can only be used in mixtures where soap gives no chemical reaction. Where soluble salts of bases other than potassium, sodium and ammonium are present insoluble stearates are formed which prevent the use of soap. Half a per cent. of soap proved itself slightly superior to both gelatine and casein while the higher percentages gave nearly complete wetting on sea-kale and a very slight wetting on the mildew. It is clear, however, that the use of soap is limited as, even at 2 % strength it proved itself inefficient when tried on the most difficult surface. The paraffin emulsions proved themselves much more potent.

The strengths tried were as follows:

Paraffin	Emulsion	1, 1
,,	,,	$1\frac{1}{2}$, $1\frac{1}{2}$
,,	,,	2, 2
,,	,,	1, 5
,,	,,	2, 1

In each case the figure first given is the percentage of soap and the

second that of paraffin. (Thus P.E. 2, 2 indicates Paraffin Emulsion, 2 % soap = 20 lbs. to 100 gallons of water and 2 % paraffin = 2 gallons to 100 gallons of water.)

P.E. 1, 1 gave superior results to 2 % of soap in that it gave better wetting of the mildew. P.E. $1\frac{1}{2}$, $1\frac{1}{2}$ was still better while P.E. 2, 2 gave perfect wetting. P.E. 1, 5, though it nearly completely wetted the mildew, was found to wet by means of paraffin instead of by aqueous solution and so, though a cheaper mixture than P.E. 2, 2 would have great danger of scorching foliage. P.E. 2, 1 stands intermediate in wetting power between 2 % soap and P.E. 2, 2 and shows the influence of introducing emulsified paraffin on the wetting properties of the liquid.

There is no object in introducing greater quantities of either paraffin or soap as P.E. 2, 2 gives perfect wetting. On the other hand the behaviour of P.E. 1, 5 and P.E. 2, 1 show that it is not possible to reduce the quantities of either without destroying the desirable qualities of the mixture. P.E. 2, 2 is, therefore, the cheapest mixture that can be used which at the same time has the highest wetting power. The value of this 2 % emulsion lies not so much in its own killing power as in the fact that it can act as a carrier, so to speak, for other fungicidal or insecticidal bodies which, used alone, would prove themselves insufficient to kill. Thus liver of sulphur, used alone, has no great controlling effect on American gooseberry mildew but, combined with paraffin emulsion, has given promising results in a commercial scale experiment undertaken by Prof. Barker and myself. In the direction of insect control it also shows promise. While dilute solutions of nicotine are without decided action on adult caterpillars or difficultly killed beetles such as *Byturus tomentosus*, the Raspberry Beetle, it has been found possible, at any rate on the small scale, to kill these by uniting the same nicotine solution with 2 % paraffin emulsion.

EMPUSA MUSCAE versus *MUSCA DOMESTICA* L.

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(With Plate XXI.)

The biological control of insect pests has received considerable attention for some years past, and success has without doubt accompanied research work along these lines. The common house fly (*Musca domestica*, L.) is more than ever now, because of additional proof furnished by more recent investigations into their responsibility as carriers of wound infections, recognised as an important source of danger to public health.

The most destructive disease, to which the house fly is subject, is that caused by the fungus *Empusa Muscae*. The question whether this fungus may be used in the control of this pest is of decided interest, and has received more or less careful attention from writers, as well as from investigators.

It has already been pointed out in an earlier paper[1] that it is an easy matter experimentally to spread the disease among flies in captivity. That is to say material from flies recently dead from the disease produced infection in others brought into contact with the spores of *Empusa*. Yet, fatal as the disease is among flies, it was for long a matter of conjecture whether it really constituted an important factor in the death of flies. No doubt the very fact of its being fatal, coupled with the number of dead flies to be seen every year towards autumn, helped to attach an importance to this disease, which a close study failed to confirm. Close observations made in places, where flies are present by thousands throughout the season, indicate that perhaps one in a thousand flies is killed naturally by the disease,—in some instances even a smaller proportion. The determination of the mortality among flies from this disease is comparatively simple, since, as a rule, the fly killed by *Empusa* is and remains attached to

[1] Güssow, H. T., "*Empusa Muscae* and the Extermination of the house fly." *Rep. Loc. Govt. Board, on Publ. Health and Medical Subjects.* New Series, No. 85, p. 10.

the substratum where it died. In the localities referred to, many thousands of flies frequented the places daily, but only from 1 to 4 at the most of newly deceased flies' bodies were observed on any one day during a period of 22 days. Hence in nature this fungus does not appear to account for the general disappearance of flies in winter.

Though I am quite satisfied that one cannot speak of this disease as the cause of an epidemic among flies, and though, endemically, it may be present every season, the fact that it is so fatal to individual flies made it desirable to experiment with the fungus with a view to spreading the disease more widely and rapidly. This might be done were it possible to grow the fungus conveniently in artificial culture, and use it in the manner of, for instance, D'Herelle's *Coccobacillus acridiorum*, pathogenic to grasshoppers.

Methods of securing artificial cultures have been tried by a number of observers, and because of some recent publications on the subject, which, perhaps, have not come to the attention of scientific workers, it will not be out of place to discuss the opinion of these writers here. In the last note which I prepared (*loc. cit.*), I briefly dealt with the investigations of Mr Edgar Hesse, on the subject of culture of *Empusa*.

The seventh report of the same Board (No. 102, 1914) contains further contributions to the subject under discussion. In the letter of transmittal Dr Newsholme, the medical officer of the Board, briefly reviews the original contributions in the report. We now take exception to the statement that the *Empusa* "is known to be the cause of much of the mortality among the house flies during the autumn months," because of careful observations on the rate of mortality. Notwithstanding my friendly criticism of Mr Hesse's 1912 work in the sixth report, his work receives much support from Dr Bernstein who was appointed by the Board "to supervise and control Mr Hesse's investigations." It must be pointed out here that Mr Hesse claimed and "proved" (!) "the possibility of obtaining from spores of *E. Muscae* derived from flies which had died of the disease, a culture which, under suitable conditions as to moisture and food material, gave a luxuriant growth in a few days, followed by the production of spores. These spores when mixed with syrup and fed to normal flies, induced in them a rapidly fatal disease, which, as judged by the naked eye and by microscopical examination, was indistinguishable from the disease found in the flies attacked by *Empusa Muscae* in the ordinary course." Mr Hesse, be it noted, refers to luxuriant growth and production of spores, but does not here state what growth or spores.

When, through the courtesy of Dr. C. Gordon Hewitt, some original material of Mr Hesse's culture was placed at my disposal, I could, on examination, find nothing else but *Mucor racemosus,* which certainly produced after a few days a luxuriant growth of the same fungus: we also observed "spores," but not *Empusa* spores. Since that time experiments have been made with spores of this universal mould fungus on living flies, but, notwithstanding an ample supply quite sufficient to fatten them, they died of starvation; but under no circumstances was death due to *Mucor racemosus,* since sound flies, kept under identical conditions minus the fungus, died about the same time from the effects of confinement. Mr Hesse solved the difficulty apparent in this instance by concluding that this fungus was polymorphic, and "that *Empusa* was merely a parasitic form of the *Mucor.*" Mr Hesse supplements this statement by the following paragraph:—

"The idea that the *Empusa Muscae* might be polymorphic now presented itself, and also that this might be the reason that its cultivation had not apparently been successful hitherto. I realised that one should not expect to produce a purely parasitic growth on a dead medium (*sic!*), but that, as in the case of other fungi when artificially cultivated, a morphological change might be expected; and came to the conclusion that *Mucor racemosus,* although a saprophyte, might, when the spore is ingested by the fly, assume a parasitic existence and produce the growth, in the tissues of the fly, known as *Empusa Muscae,* whose spores, when cultivated on artificial media, revert to the saprophytic stage."

It is regrettable to notice the author's attempt to interpret an error of his by proposing a somewhat fantastic theory. Surely before such explanation is necessary, he should be sure that what he recorded as occurring did actually occur, viz.: the growth of *Mucor* from germinating *Empusa* spores. It is so clear that it hardly needs stating that he has confused an adulteration of his "cultures" with what he thought he had produced. The use of an egg yolk medium "in thin layers in a Petri dish, sterilised by steam at 100° C. for one hour on three days in succession," clearly indicates the degree of importance attaching to the author's further results, whatever they may be.

He proceeds then by inserting bodies of flies which had died of *Empusa* during the previous autumn. Control dishes were also prepared. In five days sporangia had formed on the dishes containing the egg medium, whilst the control dishes remained quite sterile. The author, unfortunately, does not indicate whether he placed on his

control dishes dead flies, not infected with *Empusa*, but at any rate collected at a similar date (the previous autumn), which precaution one would naturally expect in controlling the results of the first experiment. It appears that he placed no flies whatsoever on his control dishes, which naturally remained sterile. Had he done so, we are prepared to predict the identical formation of sporangia observed in his experiments with *Empusa* killed flies.

Next he made transfers from his culture, sporangia forming in the secondary culture. "Meanwhile," he states, "the fungus of his secondary culture, which in every respect was identical with *Mucor racemosus*, was transferred to a syrup made of sterilised sugar and water, and put aside for experiments." How long he does not say. Nor whether the spores had time to germinate in the sugar solution in the "meantime."

Now comes the most surprising effect! In his experiments Mr Hesse uses "flies all bred from insects in confinement"; in June "the chrysalides of the greater and lesser flies commenced hatching out" (? *Musca domestica* L. the common house fly, and *Fannia canicularis*, L. the lesser house fly). In his first experiment all negative results were secured, owing apparently to absence of plum jam. Notwithstanding an earlier statement by the author that many insects became entangled in the sticky mess of plum jam, in the second experiment this was used together with, though not specifically so stated, a filter paper saturated, as in the first experiment, with a syrup in which had been incorporated spores (we take it *Mucor* spores). By the fourteenth day the first deaths were noticed; by the twenty-first day all flies were dead. "All presented typical outward (*sic*) appearances of infection with *Empusa Muscae*." No control was made in this case, he states.

In experiment three,—a repetition of No. 2,—the first deaths occurred in four days, and by the eleventh day all were dead. Cause of death is not stated.

In the fourth experiment there was a control with flies not fed with "spores," and some fed with spores from the fourth generation of *Mucor*. "The results were the same" (as which?). In the control "no deaths occurred and the flies appeared to be normal four weeks later." This is most remarkable; and, if Mr Hesse had not reported this as an actual observation, I should feel inclined to challenge the accuracy of these observations.

Cultures were then made by this indefatigable worker from "flies which had died in the cages with the naked eye appearance of infection

with *Empusa Muscae*." These resulted in a prolific growth of *Mucor racemosus*. Why, I wonder, the cautious expressions of "outward" and "naked eye" appearances? One must instinctively ask:—Was *Empusa Muscae* present, or did it only appear to be present? A statement of this most important fact is carefully avoided. Why?

Dr Bernstein thus adds his opinion: "There can be no doubt than that Mr Hesse has succeeded in producing a growth, ingestion of the spores of which results in the death of all the flies from *Empusa Muscae*." One cannot but feel greatly surprised at such a statement from the nature of the research work quoted.

After consultation with Dr Copeman and Mr Ramsbottom (of the Nat. History Museum) the investigators, Dr Bernstein states, agreed on this fact. Here I should wish to ask:—Did they agree on the fact that the flies dead from *Empusa Muscae* were the flies used, or did they merely agree that all the flies, presumably submitted for examination, were dead from *Empusa Muscae*?

Dr Bernstein now steps in the breach and tries a number of experiments. Cage 1 was not interfered with; Cage 2 received a paper saturated with sterilised syrup of cane sugar; Cage 3 received in addition a paper saturated with syrup containing the spores of *Mucor hiemalis* supplied by Mr Ramsbottom. Cage 4 contained a paper saturated with syrup containing spores of *Mucor racemosus*, "which had been cultivated on egg yolk from *Empusa Muscae* (*sic*). The cages were kept in a warm room (in whose charge, or under whose observation, we would gladly have seen stated). Abnormal changes only occurred in Cage 4. In fourteen days 75 % of the flies were dead. Mr Ramsbottom, examining one of the flies from this cage, expresses his opinion that the manifestations were identical with those of *Empusa Muscae*. No fly died in the other cages from *Empusa*.

The general conclusions are stated as follows:—"It would seem then that there could be no doubt that the deaths of the flies in Cage 4 were due to a fungus indistinguishable from *Mucor racemosus*, but which can be readily cultivated in great quantities from the bodies of flies dead of *Empusa Muscae*." This is more correct; we note "from the bodies of flies dead of *Empusa*"; what rôle did the *Empusa* play therein?

Empusa Muscae spores under no circumstances have produced in the cultures made by myself and many other workers anything else but what belonged exclusively to that fungus; certainly never anything like *Mucor racemosus*. *Mucor racemosus*, or *Mucor* resembling what

may now be somewhat loosely called *Mucor racemosus*, was readily secured from the bodies of any dead fly. We cannot, of course, doubt the correctness of the recorded observations; but we are of the opinion that if a *Mucor* proved fatal to flies in the manner described in these experiments, it must have been one of the pathogenic types or a different species altogether. We expect close diagnostic studies of the pathogenic organism would soon establish the identity. But we do absolutely question that *Mucor* developes from an uncontaminated *Empusa* spore. We are glad to note that Mr Ramsbottom has now become interested in clearing up this somewhat involved research work; and we quite agree with him that—if Mr Hesse's assumption is correct that *Empusa* is a polymorph—a fundamental biological principle would be absolutely overthrown.

The critic, they say, but assumes the rôle when he has failed in producing good or marketable merchandise in his own line. That I have assumed the critic's rôle, I may not deny: in what follows, I would play a constructive part, with what success I modestly leave in turn to my critics to say.

I wish, therefore, in concluding to record some of my cultural experiences and other observations on *Empusa Muscae*, which I have not hitherto published. This may assist in simplifying the apparent difficulties which Mr Hesse had in securing an uncontaminated growth from *Empusa* spores.

In my last paper (*loc. cit.*) I described briefly a method of successful auto-infection of flies coming into contact with flies recently dead from *Empusa*. By this means one has under one's own control the securing of fresh material of the fungus for a considerable period. By placing a fly showing the fresh belts of fungus growth on a pillar made of plasticine, this latter can be bent and twisted under the microscope into any position. When carefully adjusted one can observe the discharge of spores very readily. These fly in every direction and for distances of about seven centimetres.

Hence this fact was used for obtaining spore cultures quite easily, and uncontaminated. A series of clear coverglasses was placed at the bottom of a sterile jar, which was closed with an ordinary cork. A fly which was observed to be surely "infected"—experience having shown the incipient symptoms of infection, viz. sluggishness, increase in volume and lightening in colour of abdomen, and a peculiar reddish colour, a dirty brick red, of the "eyes"—was pinned on to the cork from below, so that, when the stopper was replaced, the fly was securely held above

the coverglasses underneath. Spores were freely shed and collected absolutely pure.

The usual methods used in spore cultures were then employed, but the results were not satisfactory. I then used for a medium various animal fats—a droplet of butter, of dripping, of lard, etc. These were exposed to the spore bombardment, but no more appreciable results were obtained. Moreover, these substances were found to be very unsuitable for microscopic use owing to their annoying refractive indices and their opacity. How much more unsatisfactory must the egg yolk medium—stone hard, no doubt, from the method of treatment given by Mr Hesse—have proved itself. I also tried sterile asparagus tips—mainly because of the known lecithin contents of this plant—but no satisfactory results were achieved on it either.

I then obtained interesting results from the use of a mineral fat, i.e. common vaseline, as used for ringing slides in hanging drops. This medium was sterile to begin with and made beautiful clear hanging drops or surfaces. A small quantity was placed with a scalpel on a clean coverglass and gently heated until evenly spread like a film over the coverglass. When exposed to spore-shedding, the number of spores desired thereon was easily adjusted by the removal of coverglasses after one, two, three or more minutes' exposure. The coverglasses were then inverted on hollow ground slides and ready for examination.

To obviate any misunderstanding, here let me remark parenthetically that, in the description following, I am not detailing the result of any one particular experiment, but am summarising the nett results of some hundreds of observations.

Germination began very shortly in every instance. The plasmatic mass which surrounds the spore when shot from the conidiophore appeared to remain in a liquid or semi-liquid form; it never showed the wrinkled appearance generally observed, and so often figured. The first symptoms of active life appeared about one hour after the spores had been shot on the medium. Never at any time did the original spore produce a germinal tube; at first there appeared something very much like a germinal tube in the shape of a small "exdentation" of the cell wall of the mother spore. This gradually elongated, but assumed, from ten to fifteen minutes later, a flask or clubshaped appearance. About an hour later it was recognised with certainty as a secondary spore, the nucleus of the first spore having taken up its position in the now forming secondary spore. As this spore grew, it, together with the mother spore, presented a dumb-bell shape. Where joined

to the mother spore the "handle of the dumb-bell" elongated somewhat, and the secondary spore also appeared surrounded by a plasmatic substance—just as did the mother spore—but in very much smaller quantity.

Immediately after the secondary spore was formed, it actually did germinate. It should have been said that in the mother cell the "exdentation" occurred on almost any position of the spherical body. But with the secondary spore, germination, as opposed to the "exdentation" of the mother cell, took place at the end towards the mother cell. Indeed what appeared at first as a rather long "handle to the dumb-bell" was later recognised as a mycelial thread, or, more correctly, tube growing into the cavity of the old spore. Naturally in many cases the secondary spore often separated from the mother spore.

The germ tube was seen to branch, and the contents of the spore to be slowly used up, while the mycelium grew in size. This produced sparse, short, stout branches at no special points. Later on a second germ tube grew from the secondary spore body; on rare occasions there were three in all. The growth made slow progress thereafter, becoming detached from its spore shell, and assuming shapes and sizes similar to the mycelial portion observed in the fat body of the fly. We have not been able to see a true tertiary spore in *Empusa Muscae* after germination took place, although peculiar club or flask shapes occasionally appeared which resembled spores; but they could not, on examination, be determined as such.

The growth remained pure all the time and made progress for 28 days on this medium. Then signs of disintegration appeared, and the mycelium became vacuoled more and more, less sharp in outline, and later collapsed. This is, so far as I know, the longest time a growth has been maintained outside a fly; but we cannot regard it as a successful culture yet. Every effort to continue growth failed, no doubt because it normally takes place in nature in the living fly body.

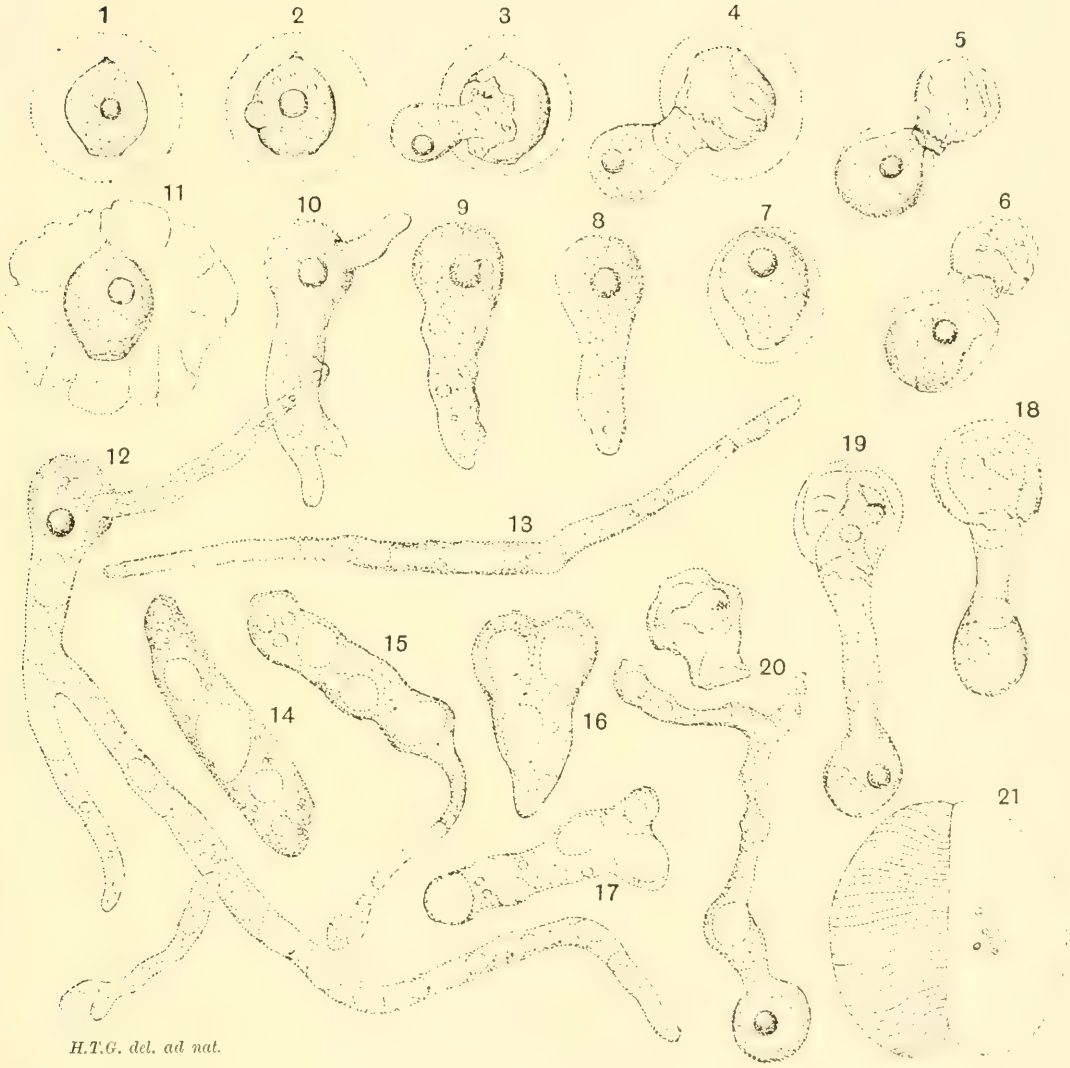
These observations clearly show that Mr Hesse's *Mucor racemosus* was nothing else but an impurity, and polymorphism does not occur in this fungus, as represented at one time by *Mucor* and another by *Empusa*.

Every other experiment to continue the development of these spores has failed so far. The absence of nutrients accounts for this; for the various ingredients tried to furnish material for continued growth did not suit the fungus. The main points, from a scientific

point of view, viz. the hibernation, the question of resting spores, so common in other species of this genus, and of artificial culture,—still remain unsolved. But, while it is realised as possible that the solution of these problems may have other than a scientific value, it appears to me from my observation that we must look, for the control of the fly problem, to other biological organisms—or remove systematically, by all necessary precautions, the insanitary conditions favourable to the breeding of this annoying and dangerous pest.

EXPLANATION OF PLATE XXI.

Fig. 1. Empusa spore shot on vaseline at 9.15 a.m.
Fig. 2. Same spore at 11.15 a.m.
Fig. 3. ,, ,, 12.10 p.m.
Fig. 4. ,, ,, 12.25 p.m.
Figs. 5, 6. Same spore, a little later.
Fig. 7. Same spore at 4.40 p.m.
Fig. 8. ,, ,, 5.20 p.m.
Fig. 9. ,, ,, 5.30 p.m.
Fig. 10. ,, ,, 6.5 p.m.
Fig. 11. Spore shot on glass slide, mass of protoplasm drying up.
Fig. 12. Same spore as before after 24 hours' germination.
Figs. 13–17. "Involution forms."
Figs. 18–20. Spore germinating into mother spore, and becoming separated from same.
Fig. 21. Proboscis of *Musca domestica* and spore of *Empusa Muscae*, relative sizes.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A BLOSSOM WILT AND CANKER OF APPLE TREES.

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(With Plates XXII—XXIV.)

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I. Introduction.

During recent years many varieties of apples have been seriously attacked by a disease which causes the wilting and death of the blossom, frequently kills the twigs, and may produce cankers on the branches. From information received at Wye College from various localities in

Kent and Sussex it is evident that the disease is increasing in intensity year by year; in some orchards and plantations it has now assumed epidemic proportions and is causing considerable loss to the fruit farmers.

The varieties which have suffered most are Lord Derby, Cox's Orange Pippin and James Grieve. A Sussex fruit grower[1] who supplied the writer with specimens gave the following list of varieties affected with the disease in one of his orchards in 1915: Duchess of Oldenburg, Worcester Pearmain, Allington Pippin, Cox's Orange Pippin (this variety very badly attacked), Early Julyan, James Grieve, Lane's Prince Albert, Lord Grosvenor, Prince Bismarck, Chelmsford Wonder, Newton Wonder, Domino and Lord Derby. In the same orchard the varieties which the owner found to be free, or practically so, from the disease were Charles Ross, Gladstone, Beauty of Bath, Lady Sudeley, Blenheim Orange, Royal Jubilee, Bramley's Seedling, Warner's King and Queen. Whether any of the varieties included in the latter list are immune is at present an open question but some of them are known to be susceptible, for Beauty of Bath, Warner's King, and Bramley's Seedling have been found elsewhere with the disease, as have also Duchess' Favourite, Ecklinville Seedling, Fearn's Pippin, Dartmouth Crab and Rival. The same grower writing in May 1915 said, the disease "is more extensive among apples than I have ever seen it before. I reckon that in some varieties one-fourth of the trusses of fruit have been ruined by it"; with reference to a similar outbreak in 1916 he wrote, "The specimens enclosed are from Fearn's Pippin which is very badly damaged. One fairly large tree blossomed all over profusely and I am sure that nineteen out of twenty of the trusses of fruit blossom have gone off like those enclosed," and in another letter he averred that "Three-fourths of my anticipated crops of Cox and Domino, and half that of some other varieties have been destroyed by the disease."

Inspection of affected orchards and plantations in Kent during the past season (1916) has shown that the experience of the Sussex grower is by no means unique, for trees with from 50 % to 75 % of the flowering spurs killed by the disease were not uncommon. On one farm visited by the writer there were thirty large standard trees, about twenty years old, of the Lord Derby variety which had produced about 300 bushels of fruit in 1914 when a little of the disease was noticed; in 1915 the disease was worse, while last season (1916) the crop was practically nil and hundreds of dead spurs recently killed were to be seen on each

[1] I am indebted to Mr Wm E. Bear, Hailsham, for the information supplied.

tree. The affected trees were in two rows; along one side of them was a row of the variety Warner's King bearing a few diseased trusses while along the other side the trees (Newton Wonder) were apparently quite free from the disease. This would appear to indicate that the Lord Derby variety is particularly susceptible to the disease and this conclusion is supported by the fact that in one large orchard where there were some hundreds of trees of this variety together with several other varieties it was possible to detect the Lord Derby trees even in winter by reason of the large number of dead spurs and twigs which they bore. In the Weald of Kent where this variety is extensively cultivated so much injury has been caused by the disease that it has been necessary in some cases to "top-graft" the trees with a more resistant variety.

Not only are well-established trees attacked, but quite young trees too are susceptible to the disease. In one case, observed in the fruit plantation at Wye College, a young cordon apple tree (of the variety Rival) was attacked through a fruiting spur situated near the middle of the stem during the first season after it was planted out; a canker developed round the base of the affected spur and killed the upper half of the tree.

The first symptoms of the disease are noticeable about a fortnight after the tree comes into flower; it will then be observed that some "fruiting spurs" of the trees affected not only fail to set fruit but the flowers and leaves round the base of the inflorescence show evidence of wilting, and, within a few days become dry and withered. Where such a truss is borne on a short spur there will be found about the middle of June a depressed, often cracked, canker-like area on the branch, around the base of the spur. In some instances the canker within a few weeks girdles the branch and so causes the death of that portion distal to the canker.

Usually no external sign of any parasitic organism is to be found on these newly killed spurs at this stage. During periods of wet weather however the dead flowers and pedicels may produce pustules bearing chains of elliptical to citriform conidia which are readily identified as of the *Monilia* type. Again if sections are made through the base of a dead truss, showing externally no trace of the fungus, and examined microscopically, hyphae are invariably to be found. When particles of these sections are placed on nutrient culture media the fungus continues to develop and may be induced to produce conidia when growing in pure culture; under these conditions too the organism is recognised as a *Monilia*.

If the dead spurs are allowed to remain on the trees until the following season the fungus appears at the surface of the spurs and over the cankered areas during the winter and spring in the form of rounded *Monilia* pustules which burst through the bark and produce numerous chains of conidia (Fig. 1).

The constant association of the *Monilia* with the Blossom Wilt and the appearance of that fungus on the diseased spurs and cankers obviously suggest that the organism is responsible for the damage done. The *Monilia* that is generally assumed by plant pathologists, in this country and abroad, to attack the apple is *Monilia fructigena* Pers. (= *Sclerotinia fructigena* Schröter). In its conidial form this is the fungus that so readily attacks the ripening apple during the late summer and often causes them to become "mummified." On such apples the fungus is usually to be seen in the form of *yellow* pustules which appear more or less in concentric circles over the diseased fruit. The form which I have hitherto always found to be associated with the Blossom Wilt was found to be quite distinct from the typical *Monilia fructigena* found on the fruit in summer and autumn, in the colour and size of its pustules, in the dimensions of the conidia and in its habit and growth on artificially prepared culture media. The pustules of the Blossom Wilt fungus are in general smaller than in *M. fructigena*, are grey rather than yellow in colour and the conidia they bear are smaller. This grey *Monilia* of the apple conforms more nearly to descriptions of *Monilia cinerea* Bon. (= *Sclerotinia cinerea* Schröter) which, according to those continental mycologists who recognise this as a species distinct from *M. fructigena*, is the form responsible for the majority of cases of Brown Rot in the "stone-fruit" trees (i.e. plum, cherry, damson etc.).

II. Historical.

In 1888 Sorauer (21) pointed out that not only was *Monilia* destructive to the apples themselves but that it could invade the woody tissue of the twigs which were in consequence killed towards their tips, and since that year frequent references to this "Zweigdürre" have appeared in continental phytopathological literature. This condition on apple trees is generally attributed to *M. fructigena*, while a similar disease on cherry trees is said to be caused by *M. cinerea* by those who recognise the two as distinct species.

Frank and Krüger (10) in 1899 make reference to an outbreak of Brown Rot on apple trees in the neighbourhood of Berlin; on the

affected trees fruiting spurs were killed soon after the flowers opened, the flowers and leaves became withered and the fungus penetrated into the bark of the branches.

One of the most valuable contributions to our knowledge of the Brown Rot *Monilias* is a paper by Woronin (23) published in 1899; in it he not only supplied evidence in favour of his contention that *Sclerotinia fructigena* and *S. cinerea* were distinct species and could be distinguished even in the *Monilia* (or conidial) condition, but also used the two forms in his inoculation experiments using, apparently, pure strains[1] of these fungi. With regard to his results when the flowers of the apple were inoculated, he found that the conidia of *S. cinerea* germinated on the stigmas and attacked the styles slightly but the germ-tubes were unable to penetrate further into the flower. The germ tubes of the conidia of *S. fructigena* on the other hand grew out into all parts of the flower and then into the pedicels and leaves, the latter gradually becoming withered. From these results he states emphatically, "Meine Experimente beweisen ausserdem, dass die Laubdürre bei den Aepfeln nur durch *Sclerotinia fructigena* verursacht werden kann."

In 1900 Aderhold (1) records having received specimens of apple twigs which, from their appearance, he believed had been destroyed by "fire-blight," the bacterial disease which ravages pear and apple trees in America. The affected shoots contained a white mycelium which when cultivated in hanging drops produced a *Penicillium*-like fructification[2] which he considered was the conidial form of a *Mollisia*. Later however (2) having found that shoots killed during the previous year bore *Monilia* pustules he attributed their death to the action of *M. cinerea* and concluded that the form previously observed was of secondary origin.

Müller-Thurgau (15) in the same year describes a similar disease of apples and pear trees but refers the causal organism to *M. fructigena*.

Aderhold and Ruhland (4) in 1905 confirmed the work of Woronin in that they found that inoculation of apple flowers with *Sclerotinia cinerea* led to a weak infection while *Sclerotinia fructigena* readily produced the death of the blossoms.

Eriksson (8) in 1913 figured and described a canker-like disease of

[1] Woronin is not quite clear on this point; on p. 18 he writes of "zahlreiche, reine, streng controlirte und möglichst variirte Impfversuche mit diesen beiden Pilzen," but whether his "reine Impfversuche" were made from cultures growing on previously sterilised media is not stated.

[2] From the description and figures it would appear that these *Penicillium*-like fructifications consisted of "sporidia" or "microconidia" of the *Monilia*, as the production of these bodies is readily induced in artificially prepared cultures of the Brown Rot fungi.

apple trees which, from the description, appears to be identical with that observed in this country. He found that the *Monilia* pustules which developed on the affected parts were grey in colour but states that he makes no distinction between *Monilia cinerea* and *M. fructigena*, and assumes that the form which appears on the fruit in autumn is a stage in the annual cycle of that form which kills the twigs and blossoms.

Broz (6) in 1913 describes the occurrence of the "Zweigdürre" at Vienna and states that late frosts favour the outbreak of the disease.

Brown Rot is also known in America where it is particularly destructive to peach trees, killing blossom, fruit and branches. The American form, though it has been generally[1] named *Sclerotinia fructigena*, is found by the more recent investigations of Matheny (14), Jehle (12), Conel (7), and Bartram (5) to conform more nearly to the descriptions given of *S. cinerea*, and is referred by them to that species.

In our own country it has been customary to refer all cases of Brown Rot of fruit trees to *Monilia fructigena*, and although the *Monilias* are probably responsible for greater losses to the English fruit growers than any other genus of fungi (since in one form or another it attacks the blossom, young fruit, ripening fruit, fruit in the store, twigs and branches), no attempt appears to have been made until quite recently to determine whether the conclusions arrived at by Continental workers hold good for Britain.

In 1903 Mr G. Massee observes (13) that *Monilia fructigena* "is most frequently seen on apples and although best known to the casual observer on the fruit occurs also on the young shoots, leaves and even the flowers." That *Monilia* is capable not only of killing the flowering spurs but may also produce large cankers on the main limbs of apple trees was recorded by Mr Salmon (16) in 1910. Since that year many specimens of Blossom Wilt and Brown Rot canker have been sent to Wye College, and it was evident that the disease was becoming a serious menace to the cultivation of some varieties of apples in certain districts, particularly in Kent.

Mr Salmon has, on several occasions[2], pointed out the serious nature of this form of Brown Rot; since preliminary examination showed that the results of investigations made abroad were not wholly in accordance with observations made on the disease as affecting apple trees in this country it was evident that it was a subject demanding further research, and as Mr Salmon himself, from pressure of other duties, was unable to continue the work, it was entrusted to the present writer.

[1] *Vide* Duggar's *Fungous Diseases of Plants*, footnote on page 187.

[2] See Bibliography on p. 203.

The investigation is still in progress but it was thought desirable that the facts already ascertained should be published in order that steps might be taken to check the further spread of the disease.

III. The Disease as observed on naturally infected trees.

(*a*) *Observations made in* 1915.

An opportunity for studying the disease under conditions favourable for close examination occurred in 1915 when a row of apple trees in the fruit plantation at Wye College was found to be attacked by the Blossom Wilt. The trees, forty-eight in number, were of the varieties Warner's King and Duchess' Favourite planted alternately and were "closely spur-pruned" bush trees about eight feet high. Detailed observation commenced early in June 1915 and the following facts were noticed:

Not one of the trees was entirely free from Blossom Wilt though two of them had but one dead truss each, while the tree which had suffered most had 132 wilted trusses of blossom or about one-third of the total number present. The affected trusses (inflorescences) were recognised by their brown and withered drooping flowers and leaves; they were often greyish since the edges of the withered leaves showed a tendency to curl inward thus exposing the hairy under surface of the leaves. That some disintegration of the tissues had occurred was evidenced by taking a spur between the thumb and finger, immediately below the insertion of the inflorescence, when it was found that, when pressure was applied, the diseased spurs were more easily compressed than was the case with healthy spurs, and wilted trusses were easily broken off at the lower limit of the year's growth. The short axis of the inflorescence was at this time quite dead and when cut across was brown throughout; microscopic examination showed the presence of mycelium in the cortex, xylem and pith, the last sometimes being almost replaced by interwoven hyphae. The discoloration of the tissues extended to the older parts of the spur particularly in the cortical region and frequently reached the branch bearing the spur.

Some of these trusses, shortly after wilting, produced *Monilia* pustules on the dead flowers during a period of wet weather but in the majority the mycelium within the tissues apparently remained sterile throughout the summer. When however some of the latter were broken off from their spurs (i.e. at the lower limit of that year's growth) and placed on damp filter paper in a large petri dish, pustules of conidia readily appeared on the exposed broken end and on the withered flowers;

in some cases well-developed chains of conidia were produced within twenty-four hours after placing the dead trusses in the moist chamber. Pustular outgrowths were observed on some of the recently produced (from infection in 1915) cankers in August but they were barren and no conidia were found on them before December.

Old dead spurs bearing conspicuous pulverulent pustules of conidia during the summer months were present on some of the trees in the row, and in those cases where cankers had been caused these too had the conidial tufts scattered over the cankered surface, particularly towards the periphery. These in all probability were the result of infection during the previous flowering season of 1914. That these dead spurs and cankers were the source of infection resulting in the blossom wilt of 1915 and that the fungus on them was the cause of the injury was suggested by the fact that the newly killed trusses were most numerous on those trees bearing the greatest number of old spurs and cankers and were most frequently met with in their vicinity. Trusses within a short distance below such spurs and cankers were particularly liable to infection; thus on one branch of a Duchess' Favourite tree a portion one foot in length bore nine dead trusses, three above and six below a dead spur with its accompanying canker on which were numerous conidial fructifications of the *Monilia*—three of the dead trusses were on the spurs immediately below the canker. (This canker is shown in Fig. 2.)

The relation between the number of dead spurs and the number of wilted trusses per tree showed, although the disease may spread from affected trees to others in the neighbourhood, it may be stated generally, particularly if the trees are well separated from each other, that the wilted trusses are infected from spurs or cankers on the same tree.

The forty-eight trees in the row had in all seventy dead spurs (some with cankers) bearing the fungus; the number of dead trusses was 1319, and since there was no other source of infection in the immediate vicinity it must be assumed that the wilted trusses of these trees were infected from the seventy dead spurs; that is to say that in that year each old spur was responsible on the average for the death of nineteen trusses of blossom. A search in the neighbourhood of a group of closely aggregated dead trusses almost invariably revealed the presence of one or more dead spurs bearing the fungus.

Infection occurred through the flowers and not through the leaves as shown by the fact that primarily the flowering trusses only are attacked. On one of the trees (variety Warner's King) was a branch

with a single dead spur, near the upper end, bearing a number of powdery pustules; all the flowering spurs, fifteen in number, on that branch below the dead spur were killed, while all the barren spurs (i.e. bearing leaves only), eleven in number, were unaffected. Barren spurs and young vegetative shoots were not attacked directly but became wilted when borne on that portion of a branch distal to a canker which had encircled the branch, or when borne on a spur which also carried one or more infected trusses.

By the middle of June it was found that where diseased trusses were borne singly on short, simple (unbranched) spurs, 0·5 cm. to 1·0 cm. in length, growing from the younger portions (5–7 mm. in diameter) of the branches the fungus had traversed the tissues of the spurs and penetrated to the branch itself producing a canker completely encircling it and causing the wilting and death of those parts beyond the canker. On older portions (to about 1·5 cm. in diameter), bearing simple spurs to 2 cm. in length, the canker at the base of each affected spur had reached about half way round the branch.

A similar condition obtained in the case of spurs killed during the previous season, and it may be stated generally that when infection had occurred through a spur to 3 cm. in length a well-defined canker was produced extending upwards and downwards along the branch for several centimetres; the lateral extension of the canker was less than the longitudinal but on those portions of the branches two or three years old it was sufficient to completely girdle them. When the spurs were longer they were often killed to the level of their insertion on the branch, but on those 5 or 6 cm. in length the fungus as a rule had failed to reach the branch and the lower portions of such spurs were still alive.

The fungus may grow downwards from the infected truss along one side of the spur while the other side remains alive and unattacked. One spur had pustules over an area extending two-thirds round it but at the apex there were two young growing apples. On another the pustules had developed over the terminal portion and along the whole of one side, the other side being still alive for about half its length as shown by a living shoot near the middle; a section across this spur showed the tissues on the one side to be brown as far as the pith, while on the other they were still green.

A few old cankers were found from which the layers of dead tissue were in process of being removed by the formation beneath them of callus which was growing over and healing the cankered surface. These cankers at this stage showed no trace of the fungus but they bore such

a general resemblance to the *Monilia* cankers that it seemed probable that they had been caused by the fungus, whose further development had been arrested. The condition of such cankers together with observations made subsequently on younger cankers suggested that the fungus did not survive after it had produced its crop of conidia. In support of this it was found that the formation of callus had already commenced beneath the bark of cankers still bearing the pustules of the fungus (Fig. 8).

The observations made in June 1915 and recorded above suggested the following hypotheses:

(1) Infection arises from conidia falling upon the open flowers.

(2) The conidia are produced on spurs killed during the previous flowering season and on cankers arising round the base of the dead spurs.

(3) When the fungus has produced its crop of conidia on a spur or canker its further development ceases and in the case of cankers the injured surface becomes healed over by a growth of callus.

That the fungus was a true parasite and able to produce both the Blossom Wilt and Cankers was definitely proved in 1916 by means of inoculation experiments with pure cultures as is shown later in this paper. The other two points were established by attaching numbered labels to affected spurs and cankers, and recording observations at certain periods. After a few weeks this revealed another interesting feature relative to the cankers. It was found that not only did the older cankers (i.e. those produced in the previous year) show no further extension of the injury produced, but that the newly formed cankers also ceased to increase in size quite early in the season; many of them had attained to their maximum size by the middle of June while in others a slight increase in the cankered area could be detected towards the end of June or early in July, but in no case did a canker show any extension after July 12. It would appear therefore that the fungus penetrates no further into the tissues of the host after some six or eight weeks from the inception of the disease.

(*b*) *Observations made in* 1916.

The observations were continued in 1916, those spurs and cankers labelled in 1915 being examined from time to time while other cankers produced during May and June 1916 were also labelled; in all a record was kept of over 100 spurs and cankers and in no case was there any evidence that the fungus was able to make any further advance into

the tissues of the trees after the end of June of the year in which infection occurs. This was found to obtain not only for the two varieties Warner's King and Duchess' Favourite, but also for Lord Derby and James Grieve of which there were a few trees with the disease in the same plantation.

On the cankers produced in 1915 conidia were first found early in December of that year, and during January 1916 a considerable number of spurs and cankers showed pustules of conidia which proved to be viable and able to germinate at the temperature of the open air. On January 27 two hanging drops of distilled water containing conidia from separate spurs were set up and placed outside in the open air. Within 24 hours many of the conidia in both drops had germinated and had produced germ tubes up to 32μ in length.

It was found necessary during the winter to remove the Duchess' Favourite trees interplanted between the successive trees of Warner's King, so observations during the flowering season of 1916 were almost confined to the latter variety; two Duchess' Favourite trees were however retained and as the disease followed the same general course on these and on a few other trees of other varieties available for examination, the following account of the disease as it occurred on the Warner's King variety may be taken as describing a typical outbreak of blossom wilt.

The withered trusses had been carefully cut off from ten trees at one end of the row during the summer of 1915, while the rest of the trees in the row were subjected to the usual pruning operations only and many dead spurs and cankers, on which pustules of the fungus subsequently appeared, were left on the trees. That there would be another outbreak of the blossom wilt on these trees was expected therefore, and from the time the flowers began to open the trees were examined frequently for the first signs of the wilt, in order that the earlier stages of the disease could be recorded, as these had not been observed in 1915.

On May 1 it was noticed that a few of the flowers on these trees were open and the rest continued to open normally during the fortnight following. Nothing unusual was observed until May 17 when one truss was found to be withered and several others were showing the first signs of wilting as indicated by the flagging of the leaves at the base of each affected truss. This wilting of the leaves is the first evident symptom of the disease, for the flowers may fail to "set" and so wither from other causes.

From May 17 to the end of that month other trusses wilted and the following sequence was recorded for one tree:

Date	Affected trusses
May 17	2 trusses wilting (leaves beginning to flag)
,, 22	5 trusses dead: 2 others wilting
,, 27	18 trusses dead: 1 wilting
,, 31	19 trusses dead.

No trusses on this tree began to wilt after May 31.

In all cases where wilting of trusses occurred subsequently to May 31 they were found to be killed not by a direct infection through the blossom but by the development of the disease in the spur or branch from infection through flowers of other trusses. In several instances a spur bore two trusses one of which became infected directly and the fungus penetrated the tissues of the spur to the level of insertion of the uninfected truss which, in consequence of the interruption to the upward flow of sap to it, wilted from desiccation.

As an illustration of the rapid progress made by the fungus during the month of May the condition of a small branch which was completely girdled by the 31st of that month may be cited; on that day the shoots and trusses borne at the nine nodes commencing at the distal extremity were as follows:

Node 1. Vegetative shoot wilting (leaves yellow and drooping).
,, 2. Barren spur wilting.
,, 3. Dead truss with canker girdling the branch and extending 1 cm. upwards and 2·5 cm. downwards.
,, 4. Dead truss with canker nearly girdling branch.
,, 5. ,, ,, ,, halfway round.
,, 6. ,, ,, ,, nearly half round.
,, 7. Living healthy truss of flowers.
,, 8. Dead truss with canker just beginning.
,, 9. Living truss.

The trusses at nodes 3, 4, 5, 6 and 8 had been killed by direct infection while the shoots at nodes 1 and 2 were wilting by reason of the girdling canker at node 3; all the spurs were unbranched and about 1·5 cm. in length; the branch, where girdled, was 0·8 cm. in diameter.

As infection probably did not occur before May 1 it will be seen that in the course of about four weeks conidia had germinated and produced mycelium which had traversed the tissues of the flowers and the spurs and had invaded the branch itself to a distance of two and a half centimetres.

On the older parts of the branches (1·5 to 2 cm. in thickness) bearing short infected spurs to 2·5 cm. in length, there was evidence, towards the end of May, that the fungus was advancing beyond the level of insertion of the spurs, by the appearance of longitudinal fissures in the bark immediately above and below the nodes. By June 7 the affected areas on the branches were slightly sunken below the general surface and extended round the nodes as shallow elliptical depressions. Further extension of the fungus appeared to be inhibited about that date and although the margins of the cankers later became more defined, no further increase occurred superficially in the majority of those examined. The bark over the affected areas gradually became sulcate, often, on the smaller branches, with distinct "oyster-shell" markings of raised lines.

Cankers which had produced the *Monilia* pustules during 1915 had been labelled and all proved to be barren during 1916, no pustules being present; no further increase of the cankered surface had occurred and in each case callus was growing over it beneath the ruptured bark. Some cankers, several inches long, were almost completely healed (Fig. 7).

Thus on trees where the disease has existed for two or more years the following results are to be observed during the summer months:

A. Result of infection during that year:
   (1) dead trusses of blossom;
   (2) cankers on the branches round the bases of the shorter spurs bearing the wilted trusses.

B. Result of infection during the previous year:
   (1) dead spurs bearing *Monilia* pustules;
   (2) cankers also bearing *Monilia* pustules.

C. Result of infection two years previously:
   (1) dead spurs with rough cracked bark, not now bearing *Monilia* pustules;
   (2) cankers more or less healed over by callus growing over the cankered surface from the margin towards the middle line.

During winter affected trees are recognised by the dried remains of the infected trusses (see Fig. 1). Flowers which fail to "set" into fruit soon fall from the tree if the spur bearing them is not diseased. When affected by the blossom-wilt *Monilia*, however, they are retained on the tree through the winter. The leaves too, of these spurs, being killed before the absciss-layer is formed, remain attached to the tree.

In December 1916 the dead spurs and cankers caused by infection in

the spring of that year began to produce *Monilia* pustules which burst through the dead bark. In the following month these became somewhat pulverulent and particles of pustules placed in water set free mature conidia. On January 24, 1917, conidia were obtained in this way in a drop of water on a glass slide when the temperature of the air, as registered by a thermometer placed on one of the trees, was 28·5° F. (= − 2° C.). The slide was placed in a damp chamber at a temperature of 6° to 8° C.; within 24 hours about 50 % of the free conidia had germinated with germ tubes to 52μ in length.

This blossom-wilt fungus apparently occurs comparatively rarely on the apples themselves, even on trees severely infected with blossom wilt. On the apple trees at Wye it has been observed on the fruit on one occasion only when it was found on a few very young apples soon after they had set. In a plantation of Lord Derby trees in the Weald of Kent there were found in January 1916 small "mummified" apples nearly covered with a *Monilia* which in cultures was indistinguishable from that found on the cankers; in the same plantation in mid-July 1915 (and again in 1916) a few young apples, about 1·5 cm. in length, were obtained, apparently killed by the same fungus which appeared in the form of numerous pustules bursting through the skin. I have not yet seen this form on fully grown naturally infected apples.

(*c*) *Comparison with other diseases producing a similar condition.*

It is sometimes impossible to distinguish, from their general appearance, between the *Monilia* cankers and those produced by *Nectria ditissima*; the latter are usually recognised by the increased rate of growth (set up in the tissues bordering the canker) which causes the branch at that place to be swollen. Young *Nectria* cankers, also, generally bear the sporodochia of the *Fusarium* stage in the summer, or the pustules if not evident readily develop when placed in a damp chamber; young *Monilia* cankers do not produce pustules until about December. Again *Nectria* cankers originate at nodes bearing unopened buds, or infection occurs through wounds; those produced by the blossom-wilt fungus arise around the insertion of infected flowering spurs.

The blossom-wilt condition bears a close resemblance to that caused by the Apple Sucker (*Psylla mali*); when the damage is caused by this insect, however, numerous cast skins are to be found attached to the under side of the withered leaves. The larva of the Pith Moth also produces a withering of the fruit buds but before the flowers themselves

expand, while in the blossom wilt caused by the fungus the effect is not noticeable until about a fortnight after the flowers open.

The disease has by some growers been attributed to late frosts; inoculation experiments (described later in this paper) carried out in a greenhouse show conclusively that the fungus readily produces the wilt in the absence of frost.

It would seem that the diseased twigs and branches bear a close resemblance to those attacked by "Fire-Blight" (*Bacillus amylovorus*) in America. Prof. H. H. Whetzel, Professor of Plant Pathology in the Cornell University, U.S.A., when he visited Wye College a few years ago, saw trees badly cankered by *Monilia*, and informed us that the general appearance of the diseased branches was indistinguishable from the condition induced by the attacks of the *Bacillus*, and, as already pointed out, Aderhold at first attributed the "Zweigdürre" of apple trees to the "Fire-Blight" organism. In this connection it may be mentioned that dead apple twigs examined by Prof. Worthington E. Smith (20) during the winter of 1898–9 were pronounced by him to have been killed by "Fire-Blight" since bacteria were found in the dead tissues. No conclusive evidence that *Bacillus amylovorus* occurs in this country has yet been published, and as old spurs killed by *Monilia* often contain bacteria in abundance, it seems probable that Prof. W. E. Smith was mistaken in his diagnosis and that the specimens seen by him were twigs killed by Brown Rot.

IV. The Blossom Wilt Fungus compared with other *Monilias* of Fruit Trees.

(*a*) *Cultural studies of Monilias found in this country.*

While circumstantial evidence obtained during observations made in the open made it very probable that the *Monilia* found on the spurs and cankers produced infection of the flowers, for confirmation of this it was necessary to reproduce the disease by the inoculation of flowers, using pure cultures. The organism has therefore been cultivated on sterilised media, and the cultures obtained have provided a means of comparing this with other forms of *Monilia* with respect to certain characters appreciable only in pure cultures.

Some thirty strains of the fungus have been cultivated in pure culture, many of them in duplicate, and, without exception, no constant differences could be detected in the various strains. Two methods of obtaining the cultures have been adopted. When the fungus is found in the

conidial stage, conidia are isolated on agar plates; the resulting sporelings which are seen to be uncontaminated are transferred to other plates and when sufficient growth has taken place sub-cultures are prepared from these. Cultures for the continued propagation of any particular strain are made on agar slants in test tubes. Strains have been obtained in this way from dead spurs, cankers and young apples; one was prepared from conidia induced on a dead truss by placing it in a damp chamber, and another from a "mummied" apple.

When the fungus is in the form of sterile mycelium only, as in the majority of freshly killed spurs and young cankers, the surface of the affected parts is gently rubbed over with cotton wool damped with absolute alcohol[1], and transverse sections are made through the diseased tissues with a sterilised razor. These are dropped into a watch-glass containing sterile distilled water and particles of the sections are then removed with flamed needles (avoiding the outer layers of the cortex) to another watch-glass of sterile water, from which they are finally transferred to agar plates. As a rule cultures which were apparently quite pure could be obtained directly in this way; sometimes, particularly in the case of spurs which had been dead for some weeks, bacterial colonies appeared immediately round the section but sub-cultures taken from the peripheral mycelium could be obtained pure. Such cultures were indistinguishable from those obtained from conidia.

For comparison with these, cultures of the Brown Rot fungi from other sources have also been prepared, e.g. forms growing on various "stone-fruits" (plum, cherry, damson, peach), on apples and on pears. Each of the strains has been started from a single conidium. In those instances where no viable conidia were present this could not be done directly and the cultures were obtained initially from sterile hyphae; the production of conidia was induced by growing on sterilised potato, and "single-spore" strains were then prepared.

I have not yet obtained the ascigerous (*Sclerotinia*) stage of any of these forms, so have been unable to compare them with the descriptions of that stage as found on the Continent and in America. The fungus which appears so generally on ripening apples is however undoubtedly the *M. fructigena* as described in Woronin's paper(23), from the large size of its pustules (usually 1·5 to 2 mm. in diameter), the colour of the pustules, which is a buff yellow, and the large size of the conidia; the latter are usually about 20μ in length but often exceed that. This species I have obtained from the apple, pear, cherry, plum and peach,

[1] 94 % alcohol has also been used with equally good results.

but, to the time of writing, only on the fruit and not on the flowers or twigs[1].

The blossom-wilt *Monilia*, as it occurs on the twigs, fruiting spurs and cankers of the apple tree in winter and spring, has pustules generally smaller (but they may reach a diameter of 1·5 mm.) and grey in colour rather than yellow, though when very powdery they may become somewhat ochraceous and are then easily mistaken for *M. fructigena*, while the conidia are much smaller, being usually 10–12μ in length and rarely exceeding 14μ. When, as occasionally happens, it occurs on young apples in summer the dimensions of the conidia are greater and this again may lead to some confusion.

The two species grow vigorously on suitable sterilised culture media and the utilisation of this property will probably afford a ready and more definite means of identifying them, particularly as cultural methods can be employed when the fungi are in the condition of sterile mycelium only. Differences in habit of growth and development of conidia distinguish them when growing on two easily prepared media, viz., prune-juice agar and steamed potato.

The medium generally used by the writer to obtain mycelial growth is a decoction of prunes (1 % stoned prunes) containing 1·5 or 2 % agar-agar. *M. fructigena* produced, on plate cultures of this medium, a flat disc of mycelium, circular in outline and growing out regularly to the edge of the plate with a margin laciniate or almost entire. The growth of the blossom-wilt *Monilia* at first resembles that of *M. fructigena* but soon shows a tendency to become lobed, the lobes being deltoid or flabelliform with narrow sinuses between them; a characteristic feature of these cultures is for growth to be arrested at about mid-way between the centre and the edge of the plate and fresh growth commences as flabelliform radiations from between the lobes. This condition has not been observed in cultures of *M. fructigena*.

Neither species produces conidia on the prune-juice agar medium although both give rise to numerous clusters of minute spore-like bodies produced in radiating chains which in the earlier stages of development resemble the fructifications of a *Penicillium* but later become dense irregular clusters. These "sporidia" were observed on *M. fructigena* by Humphrey (11) who states that they germinate and produce mycelium. Woronin figures them for *M. fructigena* and *M. cinerea* but failed to

[1] Since the present paper was sent to the press evidence has been obtained which suggests that *M. fructigena* may extend from the infected fruit into the branches and form cankers, in the case of certain varieties of soft-wooded apple trees (e.g. James Grieve); this point is being investigated.

induce them to germinate; attempts by the present writer to obtain germ tubes from these sporidia have also failed.

Of sterilised media the most suitable for the development of conidia has hitherto proved to be steamed potato, and in diagnosing a fresh strain this is at present always used. Roux's tubes are found useful for these cultures though ordinary wide test tubes are also employed. Vigorously growing mycelium transferred from an agar plate will reach the edge of the potato in four days and tufts of conidia are produced within a week. Measurements of conidia from potato cultures were taken when the cultures were 6–8 days old; if left for a longer period the conidia, particularly those of the canker strains, became overgrown by hyphae probably from their own germ tubes. The conidial tufts of *M. fructigena* in these cultures are yellow in colour and usually form distinct continuous raised zones towards the upper end of the potato, while those of the canker strains were smaller, scattered, more diffuse, and grey in colour.

An examination of the *Monilias* found on the stone-fruit trees (*Prunus* spp.) in this country led to the discovery that there are (in addition to *M. fructigena* which occurs frequently on the plum and sweet cherry) two forms both of which may be referred to *M. cinerea*, though they differ from one another culturally, and one of these appears to be identical with that form which causes the destruction of the apple blossom. The latter when growing as plate-cultures on prune juice agar is at first hyaline, but, when growth has extended for about 2 cm. from the point of inoculation, a circular band appears, olive green to dark brown in colour, approximately at 1 cm. from the centre, and later other brown zones develop. This brown coloration is more intense when the fungus is grown as agar slant cultures and appears to be favoured by the depth of the medium, for the brown zone first appears as a curved transverse band on the lower (deeper) side of the point of inoculation. The colour becomes darker and more general with the age of the culture which eventually is almost black throughout. Strains which also become brown on this medium have been obtained from the stone-fruit but whether any of these are able to infect the apple flower has not yet been determined.

Other strains, obtained from plums and sweet cherries, remain hyaline both in plate and tube cultures of prune-juice agar. Of two strains obtained by isolating conidia from a damson, one retained the hyaline appearance, the other produced a dark brown band when the two were grown side by side on the same plate.

Other differences have also been detected between the two types; thus the blossom-wilt form produces conidia less freely than the hyaline form from plums when grown on steamed potato. Again when inoculations are made on mature apples the former soon causes the fruit to become dark brown to black with conidial pustules absent or very few in number, while with the latter the dark colour is less pronounced and numerous pustules are readily developed.

Four strains have been obtained from flowers and twigs of acid (Morello) cherry trees and these on prune-juice agar produce a brown coloration which however is not so uniform as that of the strains from the apple trees.

Whether the hyaline form is less virulent than the brown is at present uncertain, and it is proposed to carry out inoculation experiments with that object in view, but it is significant that all the strains obtained from wilted trusses and cankers of the apple tree produce on the agar cultures a coloration which eventually becomes almost black.

(*b*) *Dimensions of Conidia.*

Apart from the colour of the pustules, the size of the conidia is the character most generally quoted as determining the species of *Monilia* (in the absence of the ascigerous stage) found on cultivated fruit. Of the strains of the blossom-wilt fungus at first studied only a few conidia were measured; it was soon evident however that not only did the average size of the conidia vary with the conditions under which they were produced, but also that there was considerable variation in the dimensions of conidia taken from the same pustule. The number taken therefore in order to obtain a fair average was in each case 100, for those strains obtained more recently.

It has already been stated that the *Monilia* of the apple blossom wilt has in general smaller conidia than *M. fructigena* and this difference is particularly well marked if a comparison is made of the dimensions in the two forms when each is producing pustules most abundantly under natural conditions. Thus if conidia of the blossom-wilt fungus are taken from cankers or spurs in the spring when this form is most conspicuous, the typical conidia are found to measure 11–12 × 8–9μ. *M. fructigena* on the other hand is most vigorous in late summer and early autumn when the majority of its conidia reach or exceed 20μ in length. If, however, the two are grown under the same conditions, as, for example, on sterilised potato in the laboratory the difference is less

striking, for the conidia of the blossom-wilt *Monilia* are then considerably larger than when they are produced on spurs and cankers.

Four strains obtained from dead spurs and cankers from February to April, 1916, were subsequently grown on steamed potato and in each case 100 conidia were measured[1] with the result as shown in the table. The conidia taken from the sterilised potato were measured when the cultures were 6–8 days old.

Strains of the Blossom Wilt *Monilia* from spurs and cankers of apple trees	Average of 100 conidia taken from spurs or cankers	Average of 100 conidia taken from cultures on steamed potato
Strain XI from dead spur, Feb. 1916	12·0 × 8·5 μ	19·0 × 15·0 μ
,, XII from dead spur, March 1916	11·5 × 8·5 μ	19·0 × 15·5 μ
,, XIII from a canker, April 1916	11·5 × 8·5 μ	18·0 × 14 μ
,, XIV from another canker, April 1916	11·0 × 8·0 μ	18·0 × 13·5 μ

Strain XIV was subsequently used in inoculations on apple blossom in the open, and conidia obtained from an infected flower stalk in July had an average size (for 100 conidia) of 15·5 × 12·0μ, but conidia taken during the following winter (February 1917) from a spur killed by inoculation with this same strain gave an average of 11·0 × 8·5μ which is a close approximation to that obtained from conidia taken from the original canker.

A strain obtained from young apples soon after setting (June 12, 1915) was found to have conidia the majority of which were from 17 to 20μ in length but sufficient measurements were not made for an average to be taken comparable with the above. Another strain obtained from young apples in July 1916 gave an average of 18 × 13μ for 100 conidia taken from the fruit itself, and when grown on steamed potato the average was about the same, i.e. 17·5 × 14·0μ. The other cultural characters of these two strains, e.g. habit of growth and brown coloration on prune-juice agar, and grey conidial tufts on potato, resemble those of the typical blossom-wilt strains.

It has not yet been ascertained what factors determine the output and the size of the conidia, but probably temperature and the degree of moisture have some influence. The conidia are invariably smallest if obtained in winter when the fungus is living on dead wood and bark; they are much larger when produced on young apples in the summer or on potato at the temperature of the laboratory (15°–18° C.).

Therefore when a *Monilia* obtained from an apple tree is examined with a view to its identification it is essential that the conditions under

[1] The readings were taken correct to 0·5μ and the averages were calculated also correct to the nearest 0·5μ.

which the conidia are produced be taken into consideration. The range of variation and the average size of the conidia of the blossom-wilt fungus under certain conditions is indicated in the accompanying table:

Dimensions of the Conidia of the Blossom-Wilt Monilia.

Source of conidia	Range of variation	Average
Spurs and cankers in winter and spring	7 × 5 to 20 × 13 μ (usually 10–14 × 7–9·5 μ)	11·5 × 8·5 μ
Young apples in the open in summer (June and July)	9 × 7·5 to 25 × 17 μ (usually 16–20 × 11–14·5 μ)	18 × 13 μ
Potato cultures at temperature of laboratory (15°–18° C.)	9 × 7 to 26 × 20 μ (usually 16–20 × 11–14·5 μ)	18·5 × 14·5 μ

When this fungus is growing on young apples in the open or on steamed potato in the laboratory the dimensions of the conidia approach those of *M. fructigena*, but the smaller size and grey colour of the pustules distinguish it from the latter.

Strains of grey *Monilias* on plums show a similar variation as here shown:

Strains of grey Monilias from plums	Average of 100 conidia from original source of strain	Average of 100 conidia taken from culture on steamed potato
Strain VII, mummied plum, February	11·5 × 8·0 μ	16·0 × 12·5 μ
,, VIII ,, ,, April	11·5 × 8·0 μ	16·0 × 12·0 μ
,, IX ,, ,, April	12·0 × 8·0 μ	17·0 × 12·0 μ
,, X, dead twig, June	14·0 × 10·5 μ	16·5 × 12·0 μ
,, XI, young green plum, June	16·0 × 10·0 μ	16·0 × 12·0 μ
,, XII, canker on plum tree, August	no pustules present	15·5 × 11·5 μ
,, XIII, mature plums, August	14·5 × 11·0 μ	15·5 × 11·5 μ

Strain VIII on prune-juice agar produced a browning of the medium comparable with that caused by the blossom-wilt form of apples; the others remained hyaline except Strains X and XI which were intermediate in this respect, the coloration being less pronounced (hardly perceptible in plate cultures) than in Strain VIII.

These strains from plums generally produced conidia more readily on steamed potato than did those from apple spurs and cankers; the conidial tufts of the latter were always scanty whereas the former may produce definite concentric circles of conidiophores.

Four strains have been isolated from Morello (acid) cherries and though they have not yet been closely studied they behave in cultures as the Strains X and XI from plums.

Monilia fructigena shows less variation in the size of its conidia but I have not yet found this species producing fresh chains of conidia in

the winter time; pustules examined in March and April have been barren or the conidia present were not viable, a condition observed on the Continent by Ewert(9). Three strains of *M. fructigena* of which a sufficient number of conidia have been measured gave dimensions as follows:

Strains of *M. fructigena*	Average of 100 conidia from original source of strain	Average of 100 conidia taken from cultures on steamed potato
Strain VII, mummied apple, June ...	19·0 × 12·5 μ	19·5 × 11·5 μ
„ VIII, young plums, July ...	21·5 × 13·0 μ	20·5 × 13·0 μ
„ IX, mature apple, October ...	20·5 × 13·5 μ	20·0 × 12·5 μ

(*c*) *American Strains of Monilia.*

As much attention has been given to the study of Brown Rot diseases in America it was possible to obtain specimens and cultures from that continent for comparison with those described in the preceding pages. In all, ten strains have been cultivated and examined. Apples and cultures were received from Dr G. B. Posey of the Oregon Agricultural College, a culture (from a peach) and mummified plums from Prof. L. R. Jones, Professor of Plant Pathology in the University of Wisconsin, mummified plums and peaches, together with cultures prepared from ascospores, from Mr W. A. McCubbin, Laboratory of Plant Pathology, Ontario. Thus the ten strains were from three different hosts, viz. apples, plums, and peaches, and represented three widely separated regions.

The strains are characterised by certain features common to all of them but absent from the *Monilias* obtained from material collected in this country. The most striking character peculiar to the American form is the readiness with which it produces conidia on sterilised culture media; even on prune-juice agar, as plate or as tube cultures, numerous tufts of conidiophores develop, usually within three or four days from inoculation.

When growing on plates of prune-juice agar the mycelium grows out to form a regular circular disc with an entire or sub-entire margin, resembling the growth of *M. fructigena* in its rate of development and general habit rather than that of our grey *Monilias*; the production of numerous tufts of conidiophores (often in concentric zones) in these cultures serve to distinguish it from *M. fructigena*.

On steamed potato the conidial tufts are grey and are usually so numerous that they form an almost continuous pulverulent layer over the surface. In these cultures therefore the general appearance of the

American form is very unlike that of *M. fructigena* and the conidia too are smaller than in the latter as will be seen by comparing the dimensions given below with those of *M. fructigena*.

The dimensions of the conidia of those strains where 100 conidia were measured were as follows:

	Average of 100 conidia		
	From the fruit	Agar culture	Potato culture
Strain C, mummied peach from Ontario	16·5 × 12·0 μ	14·0 × 9·5 μ	16·0 × 12·0 μ
,, D ,, plum ,, ,,	17·0 × 12·5 μ	14·0 × 9·5 μ	16·0 × 12·5 μ
,, E, culture (peach) from Wisconsin	—	14·0 × 9·5 μ	15·5 × 10·5 μ
,, F, mummied plum ,, ,,	No suitable conidia present	14·0 × 9·0 μ	16·5 × 11·0 μ
,, G, ascospore culture (peach) from Ontario	—	14·0 × 9·5 μ	14·0 × 10·5 μ
,, H ,, ,, (plum) ,, ,,	—	14·0 × 8·0 μ	13·5 × 10·0 μ

The most uniform results are obtained on prune-juice agar but as all the British strains do not develop conidia[1] under those conditions a comparison of the size of the conidia of the various forms from this culture medium cannot be made.

Growth is vigorous on the media employed; in one case conidia placed on agar germinated at room temperature (about 18° C.) producing within three days sporelings 3 mm. in diameter, and already short chains of conidia were present. On prune-juice agar plates growth takes place at a more rapid rate than in the case of the grey *Monilias* of this country, and the periodicity in rate of growth observed in the latter is absent from the American form which grows out uniformly from the point of inoculation to the edge of the plate.

In a paper recently published Bartram (5), working with strains of *Monilia* obtained in Vermont, concludes that the form in that state is *M. cinerea* but finds that it produces conidia in agar cultures; it is probable therefore that the Vermont strains resemble those received at Wye from Oregon, Wisconsin, and Ontario and that that form is the one commonly found in North America.

(*d*) *Continental Strains of Monilia.*

From our geographical situation and the frequency with which nursery stock is imported from the Continent it is natural to expect that the Monilias of this country would be closely related to, if not identical with, those of the rest of Europe, and from descriptions

[1] Very rarely have I seen an occasional short chain of conidia on plate cultures of *M. fructigena* on this medium, but no definite pustules or tufts of conidiophores have developed, nor sufficient conidia for comparative measurements.

available it seemed probable that *M. fructigena* and *M. cinerea* of the Continent were present on our own fruit trees. When it was found that cultural methods were a great aid not only in distinguishing *M. fructigena* from the grey *Monilias*, but could also be employed for the recognition of certain forms among the latter, efforts were made to obtain strains of the Brown Rot fungi from the Continent.

To the time of writing I have only succeeded in obtaining specimens from Holland, Dr H. M. Quanjer of the Instituut voor Phytopathologie, Wageningen, having on several occasions kindly forwarded mummified fruit. The fungus usually present on such material was a yellow one, in cultures indistinguishable from the yellow form of England, and there can be no doubt that this is *Monilia fructigena* Pers. One strain from these specimens was obtained from a mummified pear received in March 1916. Conidia were fairly numerous and on the average measured $19{\cdot}5 \times 9{\cdot}5\mu$, dimensions which are greater than the average size of conidia from grey *Monilias* in winter; these conidia failed to germinate when placed on agar so evidently they had not survived the winter. Particles of a pustule were teased out in sterilised water and placed on a prune agar plate; these grew out but were impure. More successful results were obtained by cleansing a portion of the skin with cotton-wool soaked with 94 % alcohol, then, raising a portion of this sterile skin with a scalpel, particles of the pulp were removed and placed on an agar plate. The resulting culture was apparently pure; sub-cultures on potato yielded conidia and the isolation of some of these afforded pure sporelings. Sub-cultures from the sporelings were then grown for comparison with other strains of *M. fructigena* and no appreciable difference has been detected either in the mode of growth or coloration; on potato the average size of the conidia was $21{\cdot}0 \times 12{\cdot}5\mu$.

One batch of fruit from Dr Quanjer received early in January 1917 included plums bearing grey *Monilia* pustules with viable conidia and a strain was isolated from each of two of the plums. Conidia, taken from the plums as received, and later obtained from potato cultures, were measured and their dimensions found to be as follows:

	Average of 100 conidia in each case	
	From mummied plums	From potato culture
Grey *Monilia* from Holland, Strain 1	$11{\cdot}5 \times 8\mu$	$16{\cdot}5 \times 12{\cdot}5\mu$
„ „ „ „ 2	$11{\cdot}0 \times 7\mu$	$16{\cdot}0 \times 12{\cdot}0\mu$

It will be seen that these figures approximate closely to those obtained, under corresponding conditions, from strains found on plums grown in

this country. The mode of growth on culture media is also similar to that of our grey *Monilias*. Neither of the strains from Holland has produced definite brown zones in tube cultures of prune-juice agar but irregular brown patches eventually appeared. They thus resemble (and are probably identical with) a form frequently found on plums and acid cherries in Kent.

I have not yet obtained diseased apple twigs from abroad.

(*e*) *Nomenclature of the various forms.*

In cultures it is possible therefore to distinguish four types of *Monilia* as found on cultivated fruit trees of the genera *Pyrus* and *Prunus*, as here tabulated:

	Prune-juice agar plate cultures	Cultures on steamed potato in Roux' tubes
(1) *Monilia fructigena*, occurring commonly on apples and plums, and frequently on sweet cherries.	Margin almost entire or laciniate; no brown coloration; conidia absent.	Conidial tufts yellow, well-developed at upper end of potato, forming raised zones.
(2) Blossom Wilt *Monilia* of the apple, also occurring occasionally on plums.	Margin with deltoid or flabelliform lobes, growth usually arrested about mid-way between centre and side of plate, and new outgrowths as flabelliform lobes develop, usually from the sinuses; olive-green, to brown, zones appear, the first usually at 0·5 to 1 cm. from the centre; conidia absent.	Conidial tufts grey, few and scattered.
(3) A grey *Monilia* frequent on plums and sweet cherries.	As above but no brown zones appear.	Conidial tufts grey, more numerous than in (2), often appearing in concentric circles round point of inoculation.
(4) American form of *Monilia*.	Margin entire or crenate; conidial tufts numerous, usually in concentric circles; brown coloration of the agar absent or appears as a peripheral band near edge of plate; growth generally more rapid than in (2) or (3) and more uniform.	Conidial tufts grey, almost covering the whole surface in a continuous layer.

As shown in the table the successive brown zones typical of the plate cultures of the blossom-wilt *Monilia* do not appear in cultures of the other forms; sometimes a brown coloration occurs in the plate cultures of the American form but in that case it commences as a peripheral band when the mycelium approaches, or has reached, the edge of the

plate. When growing as "slant cultures" in test-tubes both *M. fructigena* and the American *Monilia* usually produce a browning in old cultures, but this commences at the extreme lower end of the slant and gradually extends for some distance upwards, while in the case of the blossom-wilt fungus the first brown zone appears usually from 0·5 to 1 cm. from the point of inoculation when the culture is still quite young (often within a week).

The typical hyaline form from plums and sweet cherries remains colourless even in old slant cultures.

On the Continent, opinion is divided as to whether all instances of Brown Rot, caused by *Monilia*, on fruit trees are to be attributed to one species only (retaining for it the name *Monilia fructigena*), or to more than one. Those who favour the latter view generally assume that there are three species concerned, viz. *M. fructigena*, said to occur chiefly on the pomaceous fruits, characterised by its yellow pustules and comparatively large conidia which usually are not viable in winter: *M. cinerea*, considered to be found almost exclusively on the "stone" fruits, with grey pustules and smaller conidia which retain viability throughout the winter: and *M. laxa*, a grey form, with small conidia, occurring on apricots. Aderhold and Ruhland(4) claim to have proved that these three species are to be distinguished by their respective ascigerous (*Sclerotinia*) stages, which they describe. *S. fructigena* they obtained from mummified apples, and from the ascospores were able to develop the yellow *Monilia* stage, *M. fructigena*. That the two forms are stages in the life-history of one fungus appears therefore to be fully established. With regard to *Sclerotinia cinerea* their conclusions are less convincing. They were unable to obtain an ascigerous form from peaches, plums or cherries, so described the form, which they refer to *S. cinerea*, from apothecia (preserved in spirit) which had been found on mummified peaches and had been sent to them from America, and assumed that the *Monilia cinerea* of Europe is its conidial stage. Mummified apricots yielded a *Sclerotinia* which they described and named *S. laxa*, with *Monilia laxa* as its conidial stage.

Since, as has been shown above, the *Sclerotinia* generally occurring in America produces a conidial stage which can be readily distinguished, in cultures, not only from the grey *Monilias* of this country but also from those strains obtained by the writer from plums received from Holland, it would seem probable that Aderhold and Ruhland included in their diagnosis of the one species *Sclerotinia cinerea* two forms which are very different when grown under certain cultural conditions.

The description given of *Monilia laxa* of the apricot, as the conidial stage of *Sclerotinia laxa* applies equally well to the grey *Monilias* of other fruit. The most striking difference they give is in the size of the conidia, viz. *M. cinerea* 9·3–14·5 × 6·2–12·4 μ, *M. laxa* 12·4–23·8 × 9·3 –15·5 μ, but this difference is no greater than that shown by the blossom-wilt fungus or the Monilias of plums when growing under such diverse natural conditions as (*a*) mummified fruit or dead spurs in winter, (*b*) fresh fruit in summer.

The present writer is of the opinion from the evidence to hand that the grey *Monilias* of this country are to be referred to the continental form *M. cinerea* Bon., the *Sclerotinia* stage of which has not yet been described, unless indeed it should prove to be identical with *Sclerotinia laxa* (Ehrenb.) Aderh. et Ruhl. The blossom-wilt fungus must provisionally therefore be included under *Monilia cinerea* Bon. This species as at present delimited includes at least two forms, one which remains colourless in prune-juice agar cultures, and another which produces zones of an olive green to dark brown colour. The American Brown Rot fungus appears to be a distinct form and it may be necessary to distinguish it specifically from the European species.

V. Inoculation Experiments with Pure Cultures.

As observations in the open had indicated that in all probability infection occurred through the open flower, it was necessary that inoculation experiments should be carried out when the trees were in bloom, and the time available under natural conditions was thus limited to about a fortnight in the year. It was decided therefore that a first series of inoculation should be performed under glass in order that any information thus obtained, relative to the mode of infection, could be utilised during the same spring in subsequent experiments in the plantation. Another reason for conducting the experiment under these conditions was to eliminate the possibility that the action of frost would modify the course of the experiment.

Some difficulty was experienced in obtaining conidia in sufficient quantity to ensure successful inoculation. With the object of inducing the fungus to produce conidia readily on a sterilised substratum many culture media were tried, e.g. agar-agar media: 1·5 % agar in an extract of each of the following substances: celery, prunes, French beans, apple twigs, malt culms (also malt culms + 10 % starch), maize meal, potato. Prune-juice agar containing strips of filter paper was also used.

Woronin obtained conidia of *Monilia cinerea* on gelatine cultures prepared with apple juice or bread broth; these were tried for the apple blossom *Monilia* but without success. Nor were conidia produced on bread steamed in test tubes or on pieces of apple branches sterilised in the autoclave. The best results were obtained with semi-cylinders of tubers and roots as potato, artichoke (*Helianthus tuberosus*), carrot, mangel and parsnip. The last two produced a trace of conidia, the other three developed conidiophores in scattered tufts, and potato, sterilised either by intermittent steaming (20 minutes at 100° C. for each of three successive days) or in the autoclave for 20 minutes at 115° C., was generally used as the medium for cultures used in the inoculation of apple flowers.

(*a*) *Inoculation of apple flowers in the Greenhouse.*

For this experiment young apple trees about three feet in height were acquired; for convenience they were planted in pots early in the year (1916) and left outside until the fruit buds showed the first signs of expanding. The pots were then transferred to the greenhouse, with the result that the earliest flowers were fully expanded during the first week in April.

Conidia for the inoculations were taken from cultures, about seven days old, on steamed potato (in one instance a culture on steamed carrot was used). They were removed from the culture tube and placed on the stigmas of the flower by means of a sterilised platinum needle, which was re-sterilised in the flame of a spirit lamp after each inoculation. One flower was successfully inoculated by inserting the needle between the stamens and the styles so that the conidia were deposited at the base of the styles, but this method was not generally adopted as usually there was no space between the filaments for the insertion of conidia within the hollow receptacle of the flower.

One flower only of each inflorescence was inoculated, and of twenty-four flowers thus treated thirteen not only withered but produced a wilting of the trusses to which they belonged. The results obtained on the four varieties tested were as here shown; in one column appears

Variety	No. of flowers inoculated	No. of successful inoculations
Prince Bismarck	9	7
Lord Derby	4	2
Cox's Orange Pippin	5	2
Worcester Pearmain	6	2
Totals	24	13

the number of trusses treated (i.e. one flower of each inoculated), and in the other is the number of successful inoculations resulting in each case in the death of the whole truss.

The blossoms of the Prince Bismarck trees were the first to expand and the earliest of these were inoculated immediately after opening, some of them on the first day on which the stigmas were exposed. The flowers of the other varieties were as a rule rather older when inoculated and this suggests (judging from results as indicated in the table) that the flowers are most susceptible to infection immediately after they open. None of the untreated trusses showed any signs of wilting throughout the season except in those cases where the fungus had extended from the inoculated flowers so far as to cut off supplies to those parts not directly infected.

The results obtained from these experiments, in those instances where inoculation of a flower was followed by the wilting of the remaining flowers and also the leaves of the spur, are given below in detail to illustrate the progress of the disease from the time conidia were placed in the flowers.

All the trees were removed from the greenhouse about the middle of May and were left in the open throughout the winter.

Variety *Prince Bismarck.*

(*a*) Flower of a spur 1 cm. in length inoculated April 4 by placing conidia on the stigmas. On April 11 the styles were dark brown for a distance of 4 mm.; the whole truss was dead before the end of April and on May 5 a canker extended from the base of the spur to three-quarters of the distance round the branch.

(*b*) Flower of a spur 2·5 cm. long inoculated April 8; another truss of flowers borne on the same spur was untreated. The inoculated truss was dead by April 26 and the disease had reached the base of the other truss on that spur so that this also wilted.

(*c*) and (*d*) Flowers inoculated April 8. Both trusses were dead on April 26.

The trusses thus treated were all borne on a branch bearing in all six trusses of flowers, four of which were infected directly as shown; the fifth became infected indirectly from truss (*b*), and the sixth also wilted early in May as the result of a canker produced at the base of the inoculated truss (*d*) at the next node below. The cankers developing round the bases of the infected spurs later became confluent and by June 8 that portion of the branch bearing the trusses was cankered for

a distance of 19 cm. or from 3 cm. above truss (*a*) to 7 cm. below (*d*); the terminal portion of the branch died as a result.

The fungus itself was not observed until the first week in December when young pustules with chains of immature conidia (the conidia remained cohering in chains when particles of the pustules were mounted in water) were seen at the lower end of the canker. Later others appeared and by Jan. 9 numerous conspicuous pustules were present along the whole length of the canker and on the infected spurs. By this time the conidia were more or less pulverulent as a considerable number became free on mounting in water; such conidia were viable and many of them germinated within forty-eight hours when the slide was kept in a damp chamber at a temperature of 6° to 8° C.

(*e*) On another branch a truss was infected by placing conidia at the base of the styles of one flower on April 8. The truss wilted before the end of the month and a canker developed which had nearly girdled the branch when examined on June 8. Immature *Monilia* pustules were present at the base of the spur on Dec. 6; these were well developed by Jan. 9.

(*f*) On the same branch as (*e*) a similar truss was infected by placing conidia on the stigmas of a flower. The spur was killed before April 26, but no canker was produced on the branch. One *Monilia* pustule was found on the spur on Jan. 9.

(*g*) A truss inoculated on April 11 was dead within fifteen days. On April 27 the twig bearing it was removed from the tree and it was found that the tissues of the spur (which was 1 cm. in length) were brown to the level of its insertion on the twig and the disease was already encroaching on the tissues of the twig itself. In sections made transversely through the spur there were found numerous hyphae, particularly in the pith, and particles of the sections placed on an agar plate produced mycelial growth resembling that obtained in a similar manner from naturally infected spurs.

The exact date when the trusses of this variety first showed signs of wilting was not ascertained, but all those referred to above were dead on April 26 and had probably begun to wilt some days previously. The varieties which were treated at a later date were examined more frequently and the earlier symptoms of the disease noted.

Variety *Cox's Orange Pippin.*

(*a*) Truss of a spur 1 cm. long on a branch (0·4 cm. in thickness) bearing a terminal vegetative shoot distal to the inoculated truss.

April 19. A single flower inoculated by placing conidia on the stigmas.
,, 27. Stigmas brown to base, stamens drooping.
May 1. Truss wilting: flowers and leaves drooping, the latter curled and brown.
,, 5. Branch, bearing the truss, half girdled by a canker proceeding from the base of the spur.
,, 9. Branch three-fourths girdled: leaves of the vegetative shoot on the same branch flagging.
June 8. Canker extending to 5 cm. below the infected spur. The upper limit of the canker was not distinctly marked, but the distal portion of the branch was quite dead by this time.
Jan. 9 (1917). Well-developed *Monilia* pustules present on the dead spur.

(*b*) Truss of a spur 0·5 cm. long on a twig (0·4 cm. in diameter) bearing another flowering spur distally.

April 19. A single flower inoculated as in (*a*).
,, 27. Stigmas of inoculated flower brown and withered, stamens collapsed, pedicel discoloured to its base, sepals brown and withered. Other flowers of the same and of the neighbouring trusses showed a little browning of the stigmas on this date but in every case the stamens were upright and the pedicels and sepals were not discoloured.
May 1. Truss withered: flowers drooping, leaves curled and brown.
,, 3. The other truss (not inoculated) on same twig wilting.
,, 5. Twig completely girdled, both trusses dead.
June 8. Twig dead to the level of its insertion on the stem, but no canker had developed on the latter.
Jan. 9 (1917). One *Monilia* pustule present at the base of the spur.

(*c*) A flower inoculated on April 19 showed the same symptoms of the disease as in (*a*) and (*b*) to April 27 when the stigmas were brown to the base, the stamens collapsed and the pedicel brown throughout its length; on April 29 however it was accidentally removed, evidently before the fungus had invaded the tissues of the spur itself for the leaves were still alive in June. None of the flowers of this truss set into fruit but this was probably due to non-pollination.

Variety *Worcester Pearmain.*

(*a*) Truss of a spur 1 cm. in length on a branch 0·4 cm. in diameter.

April 19. One flower inoculated on the stigmas.

,, 27. Styles brown to the base, stamens collapsed. On a neighbouring control truss of the same age the styles were not brown and the stamens were upright.

,, 29. Flowers and leaves of the truss drooping.

May 1. Truss withered, leaves brown and curled.

June 8. Canker present on the branch, nearly girdling.

Dec. 6. Young pustules (bearing very short chains of immature conidia) appearing.

Jan. 9 (1917). Conspicuous *Monilia* pustules present on the canker and dead spur.

(*b*) Truss of a spur 1·5 cm. in length on a branch 0·5 cm. in diameter.

April 19. One flower inoculated on the stigmas.

,, 27. Styles of inoculated flower brown, some of the stamens collapsed; these features were not present on a neighbouring control truss.

,, 29. Flowers and leaves of the truss drooping.

May 1. Truss withered, leaves brown and curled.

June 8. Canker present round the base of the spur, girdling the branch and extending 2·5 cm. below the insertion of the spur; that portion of the branch distal to the canker was dead.

Jan. 9 (1917). Several *Monilia* pustules present on the dead spur.

Variety *Lord Derby.*

On this variety the inoculations which resulted in wilting were made on trusses borne on short spurs at nodes on the main stem of a young tree. The flowering spurs were four in number and inserted at successive nodes, the stem being 0·6 to 0·7 cm. in thickness in this region. The uppermost truss (*a*), borne on a spur 2 cm. in length, was inoculated; at the next node below was an untreated control truss (*b*); the third (*c*), on a spur 1 cm. long, was inoculated while the fourth (*d*) was another control. Within a few centimetres of (*a*) the stem divided into three branches all bearing leaves only.

April 19. The first flower to open (i.e. in the centre of the inflorescence) of each of the two trusses (*a*) and (*c*) was inoculated by placing conidia on the stigmas.

April 27. The styles of the inoculated flowers were withered and brown to the base and the stamens had collapsed. In the corresponding flowers (of the same age) on the control trusses the styles were not discoloured and the stamens were upright.

,, 29. All the flowers of the inoculated trusses were drooping and the leaves were beginning to wilt; the stamens of the untreated flowers were however rigid (not collapsed with distorted filaments as in the two inoculated flowers).

May 1. All the leaves of trusses (*a*) and (*c*) were by this time curled, brown and withered: the stamens of the non-inoculated flowers were still rigid. (This stage is shown in Figs. 3 and 5.)

,, 5. From truss (*c*) the disease had traversed the tissues of the spur and was invading the main stem as shown by a slight sinking of the bark at the base of the spur and extending about half the way round each side; truss (*b*) was just beginning to wilt, indicating that the transpiration current was being interrupted, and that the vessels of the xylem were attacked.

,, 10. The canker at truss (*c*) had almost girdled the stem and extended 0·5 cm. upwards and 1·5 cm. downwards from the base of the spur; the cankered area was indicated by a distinct wrinkling of the bark which was also slightly depressed below the general level.

,, 11. The stem was now completely girdled; the lowest branch distal to the affected spurs began to wilt.

,, 19. The leaves of the other two branches above the spur were drooping and turning brown.

June 2. A canker had also developed from truss (*a*) so that by this date the two cankers had united and together they extended along the stem for a distance of 10 cm., i.e. from 4 cm. above (*a*) to 6 cm. below (*c*); truss (*d*) however was still alive. Towards the upper end of the canker there was a series of dark lines more or less parallel to one another giving a zonate appearance. That portion of the tree above the infected spurs was by this time quite dead. (This stage is shown in Figs. 4 and 6.)

Dec. 6. Young pustules were bursting through the bark at the lower end of the canker; the immature conidia remained cohering in chains when mounted in water.

Jan. 9 (1917). Numerous conspicuous pustules were present along the canker and on the infected spurs; the conidia were more or less pulverulent and a considerable number became free on mounting in water. Such conidia were viable for many of them germinated within 48 hours in distilled water at a temperature of 6° to 8° C.

(*b*) *Inoculation of apple flowers in the Plantation.*

The experiments carried out in the greenhouse furnished definite proof that the fungus is a true parasite under favourable circumstances. It was desirable however that similar experiments should be performed in the plantation in order to obtain evidence that the disease could be induced in a similar manner on trees growing in the open.

Early in May 1916 inoculations of apple flowers in the plantation were made for comparison with the results obtained under glass. The flowers of the variety Warner's King began to open about May 1 and as this variety was known to be susceptible to the disease it was selected for the experiments.

As before, the conidia were obtained from cultures, about a week old, growing on steamed potato, but the method adopted in the actual inoculation of the blossoms was slightly modified, in order to avoid any injury to the stigmas that might occur when using the platinum wire in transferring the conidia from the cultures. The steamed potato, with the fungus, was removed from the culture tubes (using sterilised instruments) and placed in sterilised petri dishes; small portions bearing tufts of conidiophores were cut off with flamed scalpels and placed in another sterile petri dish which was taken direct to the plantation. In each case only one flower of each truss was inoculated and the inoculation was made by removing from the dish, on the point of a sterile needle, a particle of potato bearing conidiophores and bringing the conidia in direct contact with the stigmatic surfaces, so that the needle itself in no case touched the stigmas. The selected flower of the truss was marked, for identification and comparison with the rest, by a few inches of white cotton tied loosely round the flower.

Variety *Warner's King.*

Results of inoculations:

(*a*) May 5. One flower of a truss inoculated on the stigmas.
,, 11. One style of the inoculated flower brown to the base.
,, 18. The stamens of this flower had collapsed, while those of

other flowers of the inflorescence were upright. On this date the treated flower was accidentally removed during the examination and the disease made no further progress, the spur remaining alive throughout the summer.

(*b*) May 5. One flower inoculated, borne on a spur 2 cm. in length.
,, 11. One style brown in the middle.
,, 18. Stamens collapsed (stamens of the other flowers of that truss upright).
,, 20. Flowers and leaves of the truss wilting.
,, 29. The bark round the base of the spur beginning to crack.
Oct. 19. The disease had not extended further; the spur was dead to the base and the bark on the branch was fissured just below the insertion of the spur but there was no definite depressed cankered area.
Jan. 10 (1917). Conspicuous *Monilia* pustules present on the dead spur.

(*c*) May 5. One flower inoculated, on a branched spur bearing two trusses.
,, 11. Two styles brown to the base, two others brown for about half their length.
,, 17. The leaves round the truss wilting.
,, 18. *Monilia* pustules present on some of the dead pedicels.
Oct. 19. The infected half of the spur was dead to the base, a distance of 3 cm.; the rest of the spur, including the other truss, was alive.
Jan. 10 (1917). *Monilia* pustules present on the dead spur.

(*d*) May 5. One flower inoculated, on a branched spur bearing two trusses.
,, 18. Stamens of inoculated flower collapsed.
,, 20. Truss wilting.
Oct. 19. Infected half of the spur dead to the base with a canker two-thirds round the main axis of the spur; the rest of the spur was alive.
Jan. 10 (1917). *Monilia* pustules present on the dead portion.

(*e*) May 5. One flower inoculated, on a spur 5 cm. in length.
,, 22. Flower brown to the base of the pedicel.
,, 25. Leaves round the base of the inflorescence drooping.
,, 29. Truss quite withered, leaves brown.
Oct. 19. Spur dead for a distance of 5 cm.

Nov. 28. The spur bore several *Monilia* pustules with chains of conidia; the latter were not pulverulent and evidently immature.

Jan. 10 (1917). Numerous conspicuous pustules present along the whole length of the spur; conidia more or less pulverulent (a large number becoming free on mounting particles of the pustules in water).

Although the conidia were obtained when the temperature of the air was below 0° C. many of them germinated within 48 hours, in a drop of water kept at 6° to 8° C.

(*f*) May 5. One flower inoculated of an unbranched spur 2 cm. in length.

,, 16. Flower discoloured to the base of the pedicel.

,, 18. Leaves at the base of the truss drooping.

June 2. Bark on the branch cracking immediately above and below the insertion of the spur; a slightly depressed area extended one-quarter round the branch.

,, 26. . Canker now extended half round the branch.

Oct. 19. The canker had not extended any further round the branch; it was however quite well defined but at this date bore no *Monilia* pustules.

Dec. 1. Immature pustules (conidia not pulverulent) present along the whole length of the spur and on the canker.

Jan. 10 (1917). Pustules more conspicuous: conidia more or less pulverulent; many germinated in distilled water within 48 hours at 6° to 8° C.

A flower on each of three other trusses was also inoculated; the three flowers died but became detached about May 20 and the disease did not extend into the axis of the inflorescence. The inoculation of three flowers on May 10 and five on May 11 did not produce any wilting of the trusses and it may be that in these cases the flowers were too old for successful infection, although at the time of inoculation the stigmas appeared receptive and showed no discoloration. These results correspond to those obtained in the greenhouse where, as already shown, the inoculations were most successful on those flowers which had recently expanded.

It will be observed that in every case where inoculation with the fungus was followed by the death of the truss, *Monilia* pustules appeared on the cankers and dead spurs during the succeeding winter. Usually

they began to burst through the bark during December and many well-developed pustules with viable pulverulent conidia were to be seen by the middle of January.

Although it has been noticed that in some cases spurs showing the typical symptoms of the Blossom Wilt in the summer failed to develop *Monilia* pustules in the winter, this condition is exceptional, and where cankers have been formed these invariably have produced the conidial stage of the fungus. The results of the artificial inoculations too demonstrate that a spur showing the wilt condition in summer is almost certain to produce pustules of conidia before the following spring. The danger of allowing such spurs and cankers to remain on the trees until the next flowering season is obvious.

(*c*) *Inoculation of Twigs through Wounds.*

Twigs on trees of the Newton Wonder variety, which is known to be susceptible, were inoculated with mycelium from a plate culture of the same strain which produced wilting of the blossom in the inoculations made on the Warner's King variety. A Λ-shaped cut was made through the bark, the triangular portion turned back, and agar bearing vigorously growing mycelium was placed between the wood and the bark; the latter was then gently pressed back and the wound covered with sterile tinfoil which was secured in place by means of raffia. Three wounds were treated in this way and three others, as controls, were not inoculated. The inoculations were made on May 30, that is, at the time when cankers were in process of development on trees infected through the flowers. When examined some months later all the wounds were covered with callus and no trace of canker was to be found on any of them.

The result suggests that the fungus does not readily (if at all) produce cankers by infection through wounds on the branches, and agrees with observations in the open where, so far as my own experience goes, a canker produced on an apple tree by this *Monilia* invariably originates in a spur that has been infected through the flowers.

(*d*) *Inoculation Experiments on the Fruit.*

It has already been pointed out that the *Monilia* which causes the Blossom Wilt may occur on the young apples. It will also grow readily on apples approaching maturity and on ripe apples after picking, as artificial inoculations have proved, although under natural conditions instances of its occurrence on the mature fruit appear to be rare for

the writer has not yet met with such cases even on trees seriously affected with Blossom Wilt.

Apples of the varieties Warner's King, Newton Wonder and Bramley's Seedling were inoculated on August 10 and 11 by placing mycelium from a plate culture in wounds made with a sterile scalpel. Unfortunately the apples became attacked by ants and all fell to the ground after a few days; the experiments however continued long enough to show that the fungus rapidly produced a brown rot appearing within four days as a discoloured area 1·5 to 3 cm. in diameter round each point of inoculation.

The experiments were then continued on apples of the Bramley's Seedling variety, which were brought into the laboratory and inoculated with a strain isolated from a canker. The first inoculations of this series were made on Aug. 17 and the experiments were repeated at intervals throughout September and October. For comparison, other apples were inoculated with *Monilia fructigena*, using a strain obtained from a plum, and with a hyaline form of *M. cinerea* also isolated from a plum. In some cases *M. fructigena* and the canker strain were placed in wounds made on opposite sides of the same apple, while in others the hyaline strain of *M. cinerea* and the canker form were grown on the same apple. Under these conditions it was found that each of the three forms produced a rot which extended approximately at the same rate for all, i.e. 2·5 to 3·5 from the point of inoculation in seven days. The canker form of *Monilia* however seldom produced pustules of conidia; on some of the fruit a few scattered tufts of white barren hyphae developed but on others no pustules were produced. On the other hand the strain of *M. fructigena* used in these experiments freely produced large yellow pustules more or less in concentric circles. The hyaline form of *M. cinerea* also developed conidia readily but on smaller grey pustules which were usually fairly numerous.

The skin of those apples inoculated with the canker *Monilia* rapidly assumed a dark brown shade over the affected area, which gradually became black. This nigrescence was to be detected towards the centre of the discoloured area about a week after inoculation; it gradually extended over the surface until the whole was black. The other two strains also produced some blackening particularly in the later experiments (i.e. on the more mature fruit) but not so readily. These differences were most striking in those cases where two forms were growing together on the same apple by inoculations at opposite sides. For example, two apples were inoculated on Aug. 17 and two others on

Aug. 21 by placing mycelium from an agar plate culture in wounds on opposite sides using the canker *Monilia* on one side and *M. fructigena* on the other. The result was the same for all four; that half of the apple infected with *M. fructigena* produced numerous large yellow pustules and remained brown, while the side infected with the canker form became quite black in from three to four weeks and developed no pustules at all or from one to three minute barren tufts of hyphae. The side inoculated with *M. fructigena* became shrunken at a much more rapid rate than the other.

Similar experiments were performed about the same time using the canker-producing *Monilia* and the hyaline *M. cinerea* strain. Again that side of the apple infected with the former soon became black and bore few or no pustules, while the opposite side produced, as a rule, numerous greyish pustules and remained brown for some weeks, becoming however gradually darker until it was almost black; as before the shrinking of the skin was most pronounced on the pustular side.

Later other apples of the same variety were inoculated using the same three strains of *Monilia* but infecting each apple with but one of the three. The results conformed with those obtained previously; those apples infected with *M. fructigena* became almost covered with large yellow pustules often becoming confluent, those with the hyaline strain of *M. cinerea* produced smaller greyish pustules, while those with the canker form remained sterile or produced a few scattered tufts of aerial mycelium usually sterile.

The apples used in these experiments were such as showed no apparent injury before inoculation; they were obtained from trees growing in the College plantation and taken immediately to the laboratory and inoculated. As the crop was picked during the first week of October subsequent inoculations were made on apples (of the same variety) from the fruit-storage shed. On such fruit the results were practically as before except that the barren hyphal tufts produced by the canker-strain were rather more numerous than in previous experiments. This was probably due to small abrasions caused during the operations of picking and storing or to minute cracks produced in the skin on drying, thus allowing hyphae to grow out into the air. But even then the difference between these results and those obtained with the other two forms of *Monilia* was still conspicuous.

Whether similar results are to be obtained with other varieties of apples has not yet been determined but it appears evident that the *Monilia* causing the blossom wilt and canker of apple trees produces

conidia on the ripening fruit much less readily than *M. fructigena*, and this probably accounts for its inability to establish itself on the mature apples, for the chief sources of infection from this fungus (i.e. the pustules on the cankers and spurs killed in the previous season) have shed most of their conidia and are becoming desiccated at the time when the apples are reaching maturity. As indicated earlier in this paper the newly formed cankers do not produce conidia until long after the fruit is picked.

The blackening of the skin of apples produced by the blossom wilt fungus may also be caused by *M. fructigena* and by the hyaline grey *Monilia*, particularly on the mature fruit, but the result takes place much more gradually. Black apples with few or no pustules are frequently found among stored fruit; this condition appears to be brought about by *M. fructigena* which has invariably been isolated by the writer from such apples obtained from fruit growers in Kent.

Spinks(22) finds that a similar "Black Rot" of cider apples is also to be attributed to *M. fructigena*.

VI. Control Measures.

It is evident that cutting away the dead spurs and cankers removes the chief source of infection, and an experiment, carried out on the row of trees of the Warner's King variety referred to in preceding pages, has demonstrated that where this can be done thoroughly the results are highly satisfactory.

Ten trees at one end of the row were carefully pruned by the writer on June 15 and 17, 1915, and so far as could be seen at the time every withered truss was cut away until all the dead discoloured (brown) tissues of the spur were removed. In those cases where cankers had developed on the branches the operation involved the removal of dead bark and wood in those places, until a clean cut, showing healthy tissues only was made. The trees were not treated in any other way and the wounds made by the pruning knife were left exposed. In all over 220 dead spurs were removed from these trees. When these ten trees were examined in the summer of 1916 it was found that five were quite free from the disease, one tree had but one dead truss, three had two each, and one had six dead trusses; a search on the last mentioned tree however revealed the fact that two dead spurs had been overlooked during the pruning operations and these now bore a number of *Monilia* pustules.

The rest of the trees (fourteen in number) in the same row had been

pruned in autumn in the usual way and no special instructions with regard to the disease has been issued to the men entrusted with the work. The consequence was that although a certain amount of "dead wood" had been cut away a considerable number of spurs and cankers, which subsequently produced the conidial stage of the fungus, remained on the trees. In some cases a dead spur had been cut off close to the branch but the diseased tissues of the canker round the base had been left and later the fungus appeared there. In the spring of 1916 it was found that every tree bore the fungus in its infectious (conidial) condition, the number of dead spurs and cankers with pustules varying from one to twenty-five per tree with an average of eight. The number of wilting trusses on these trees in May 1916 was 159 and varied from two to twenty-nine per tree; these numbers would probably have been considerably higher but for the fact that the trees had produced that year exceptionally few trusses of bloom. On several trees however more than one-fifth of the trusses wilted and in one case more than half of those present were killed.

Thus on those trees from which all dead spurs had been removed (omitting the one on which two had been overlooked) the number of wilted trusses was reduced, on the average, to less than one per tree, the infection in these cases being doubtless due to air-borne conidia from the infected trees in the rest of the row, while those trees on which infected spurs were left had an average of eleven wilted trusses per tree.

The ideal mode of treatment would be the removal of diseased trusses immediately after they first show signs of wilting; this would involve examining the trees three or four times at intervals of about a week between one examination and the next, all wilting trusses being removed and the spurs cut back until all brown bark and wood is removed, the first of these operations to be done about fourteen days after the earliest flowers open. This method would prevent the development of cankers which are not only much more troublesome to remove than the spurs themselves but it would prevent the girdling and death of the small branches.

As such measures would usually be impracticable except in small plantations of bush trees where the disease has not yet become rampant, an alternative would be to prune off all diseased spurs and cut out the cankers as occasion permitted during the summer. This work should be done as early as possible for the dead trusses with their withered brown leaves are easily distinguished from the healthy ones and clearly

indicate where pruning is necessary. If the operation is left over until the winter or spring very careful search is necessary to avoid overlooking some of the dead spurs and cankers, although even in winter the diseased spurs are often easily recognised from the fact that at leaf-fall the leaves and remains of flowers on such spurs still remain on the trees, and the pedicels and petioles may still be found on them in the spring.

When, owing to difficulty in obtaining the necessary labour, the removal of the spurs and cankers cannot be carried out before leaf-fall it may be done any time during the winter, but it is imperative that all diseased parts be cut off before any of the flowers begin to open.

It is important to emphasise the fact that the operation whenever carried out must involve the removal of all brown and dead wood and bark. Thus it is not sufficient merely to break off the withered trusses. In one experiment six withered trusses were broken off from their spurs early in June 1915, at the base of that season's growth, the spurs being then labelled for future reference; in April 1916 four of these bore *Monilia* pustules.

As already shown the disease occasionally appears on the young apples causing them to become dry and withered, and such "mummified" apples may be retained on the trees until the following year. Since it is impossible to distinguish, without close examination, between this form of Brown Rot and *Monilia fructigena* when occurring in this way, it is necessary that all "mummies" be picked and destroyed during the winter, especially as *Monilia fructigena* itself is the cause of a serious fruit rot.

The cutting out of all affected parts before the blossoms open is the only treatment that can be recommended with confidence until further investigation is carried out. Owners of large standard Lord Derby apple trees have in some cases found, however, that to cut out all the dead trusses (which in serious attacks often number some hundreds per tree) is not practicable especially when skilled labour is difficult to obtain[1]. In those cases where the disease is very severe, "top-grafting" with a less susceptible variety is to be recommended.

Whether spraying is of value in controlling the disease is at present uncertain. As infection takes place through the open flower the use

[1] Our Sussex correspondent writes: "To cut off and burn the millions of diseased spurs and shoots on apples and plums is quite impracticable here."

"Millions" is probably no exaggeration in the case of large orchards, for the writer has counted over 130 wilted trusses (or about one-third of the whole) on a small bush tree, and has seen large standard trees where the proportion of dead to living trusses was even higher.

of a "cover-spray" to protect the stigmatic surface is out of the question, therefore a wash to be effective must be applied before the flowers open and must be capable of destroying the powdery conidial stage, or at least must prevent the conidia from falling during the period throughout which the flowers are open and receptive.

Experiments have been tried in the open with the Lime-sulphur Wash, which is frequently recommended for cases of Brown Rot, but no favourable results have been obtained. "Winter-washing" with Lime-sulphur failed to check a serious outbreak of the disease in the following spring. Facilities for testing the value of Lime-sulphur as a summer spray were kindly offered by Mr P. Manwaring[1] of Horsmonden, Kent, who permitted a plantation of Lord Derby trees (four rows each with thirty-two trees), which had had a severe attack of the Blossom Wilt in the previous year, to be used for experimental purposes. Three rows were thoroughly sprayed with Lime-sulphur at "summer strength" (s.g. 1·01) immediately before the flowers opened, the fourth row remaining untreated and kept as control. In May all the rows showed a serious attack of the Blossom Wilt and no difference in intensity could be detected between the unsprayed row and the rest; many of the latter had 50 % or more of the trusses killed by the fungus, and the numerous *Monilia* pustules on the dead spurs were apparently uninjured by the spray fluid.

This result is doubtless due to the fact that such a liquid as the Lime-sulphur solution is unable not only to penetrate but to adhere to the powdery pustules. Experiments carried out in the laboratory showed that Lime-sulphur solution even when applied in the form of a very fine spray with an atomizer immediately ran off from the pustules which appeared totally unaffected by the treatment.

Bordeaux Mixture applied similarly with an atomizer adhered a little more readily but the majority of the pustules were not covered by the spray.

An Ammonium Sulphide solution containing soft-soap, as recommended by Dr Eyre and Mr Salmon[2] for use on the conidial stage of the *Erysiphaceae*, was also tried in the same way. This wash did wet the pustules which in consequence became brown on the surface and lost their pulverulent appearance. The pustules themselves were not

[1] I take this opportunity of thanking Mr Manwaring for the facilities offered for investigating the disease in his plantations.

[2] Eyre, J. Vargas and Salmon, E. S. The Fungicidal Properties of Certain Spray Fluids. *Journ. Agric. Science*, Vol. VII. pp. 473-507.

killed and conidia placed in hanging drops germinated readily. It would seem however that the surface layers were killed and the question is whether this would be sufficient to prevent the fall of the conidia during the critical period when the flowers are open if the spraying were done as late as possible but before the flowers expanded. It is proposed to carry out experiments in the open to test this point.

In conclusion I desire to thank Prof. L. R. Jones (Wisconsin), Dr G. B. Posey (Oregon), Mr McCubbin, M.A. (Ontario), and Dr Quanjer (Holland), who have kindly sent specimens of mummified fruit or cultures from abroad, also Dr Pethybridge and Mr J. M. Hector, B.Sc., who sent mummified fruit and diseased branches. I am indebted also to Mr Salmon (head of the Mycological Department at Wye College) whose advice and criticisms throughout the investigation have been invaluable.

Summary.

1. A "Blossom Wilt and Canker" of apple trees, produced by a species of *Monilia*, is causing great loss to fruit growers in the south-east of England.

2. Infection takes place through the open flowers; the fungus invades the tissues of the flowering spur, thus killing the inflorescence and the leaves of the spur; the disease may reach the branch and produce a canker.

3. Spurs killed during the summer, together with the accompanying cankers, produce pustules of conidia during the following winter and spring; these conidia, falling on the flowers as they open, give rise to a new outbreak of the blossom-wilt disease.

4. When a canker has shed its crop of conidia it becomes covered with callus which eventually heals the lesion.

5. Inoculation of apple flowers with conidia from pure cultures of the fungus resulted in the death of the inflorescences and the spurs; in some cases cankers were produced. Conidia-bearing pustules of the organism appeared on these dead spurs and cankers during the following winter.

6. The causal organism is a grey *Monilia* easily distinguished from *M. fructigena*; at present it is to be referred to *Monilia cinerea* Bon.

7. On culture media the habit of the fungus is different from that of the grey *Monilia* (also referred to *M. cinerea* by American workers) which is commonly found in North America.

8. The disease may be kept in check by cutting out all dead spurs and cankers before the flowers open; on the first appearance of the disease all wilted trusses and dead spurs should be promptly removed. Spraying operations can be efficacious only when they kill the conidial pustules or prevent them from shedding their conidia during the flowering period.

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DESCRIPTION OF PLATES XXII—XXIV.

Fig. 1. Portion of cankered branch (var. Lord Derby) showing *Monilia* pustules; condition as seen in winter. (Photographed April 8, 1916.)

Fig. 2. Dead spur and canker bearing *Monilia* pustules, with neighbouring flowering spurs; the wilted trusses below the canker were probably infected by conidia falling from the spur and canker; condition as seen in summer. (June 4, 1915.)

Fig. 3. Four trusses on the main stem of a young Lord Derby apple tree; the first and the third (from above) were each inoculated, from a pure culture of the fungus, on a single flower. Result fifteen days after inoculation—both inoculated trusses are dead.

Fig. 4. As in Fig. 3 but four weeks later; the stem is cankered above and below the two inoculated trusses.

Fig. 5. The stem of the infected tree at the time that the photograph shown in Fig. 3 was taken; the four trusses there shown are to be seen immediately below the branch on the right.

Fig. 6. As in Fig. 5 but taken four weeks later, i.e. on the same day as Fig. 4 was obtained. The leaves on the branches above the canker are wilting.

Fig. 7. A canker produced by a natural infection in 1914; it bore *Monilia* pustules in 1915, but when photographed in June 1916 was barren and was being covered over by callus.

Fig. 8. The canker shown in Fig. 2 seen in transverse section (× $2\frac{1}{2}$). The development of callus at the sides of the canker has already commenced at this stage.

(Figs. 1—4 and 7 are $\frac{2}{3}$ natural size.)

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Fig. 2

Fig. 1

Fig. 4

Fig. 3

Fig. 5

Fig. 6

Fig. 7

Fig. 8

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